Peri- and postnatal predictors for adverse neurodevelopmental outcome

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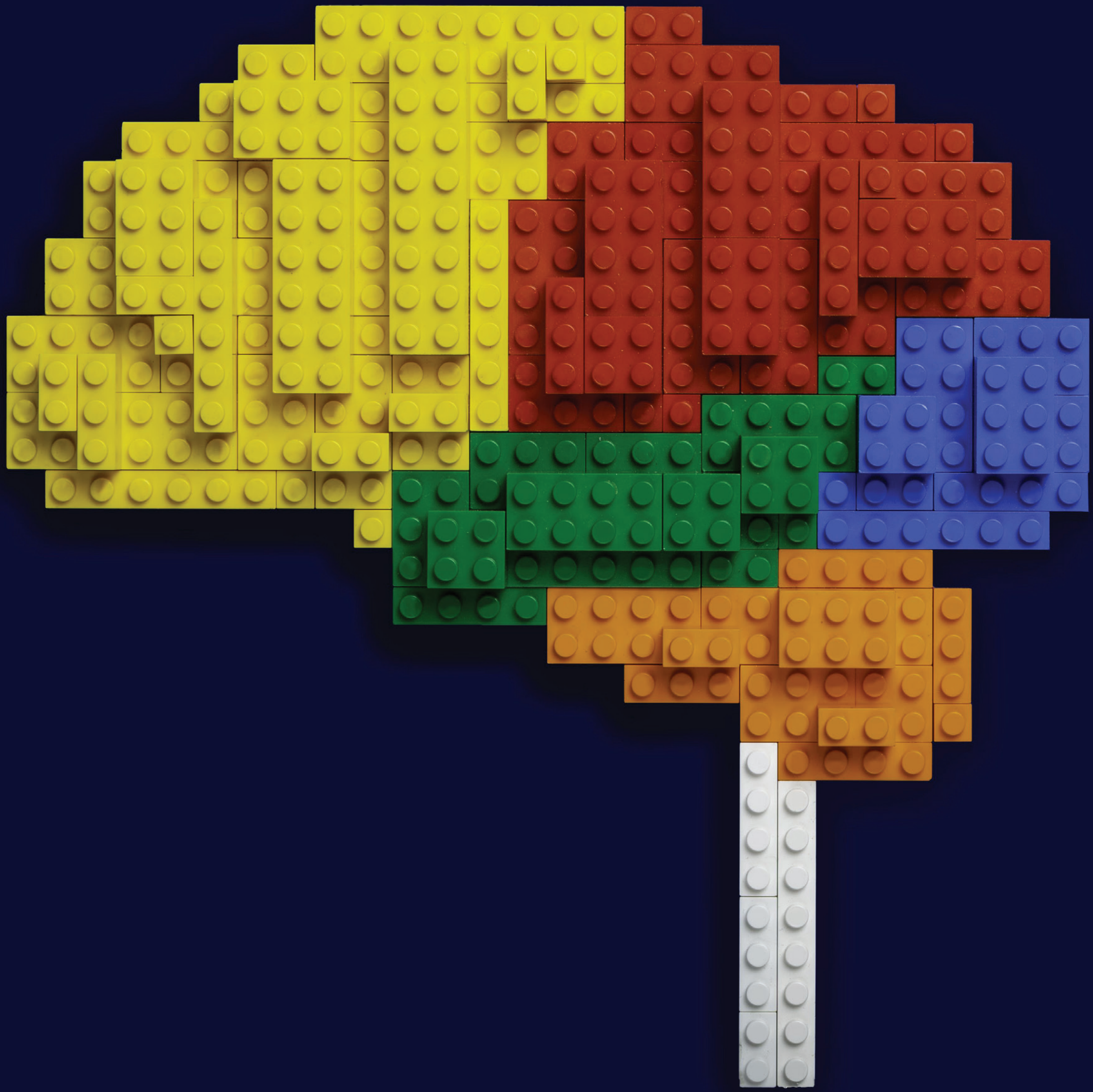

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Peri- and postnatal predictors for adverse neurodevelopmental outcome

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Chapter 1

General introduction

Over the past decades neonatal intensive care has improved remarkably. Where initially survival was the primary concern, mortality rates have decreased substantially. Long term neurodevelopmental outcome emerged as an important outcome parameter in neonatal intensive care. Insight in pathophysiology of morbidity in preterm and term infants improved over the years, and makes us understand why complications or adverse events occur. These insights might provide tools to intervene and prevent these adverse effects and thereby improve long term outcome.

Neuro-ontogenesis

Throughout gestation the brain develops rapidly. The first cortical layer formed is the preplate, the later subplate. The subplate is a transient layer, which contains many synapses. This plate forms the precursor of later, more enduring connections for the thalamocortical neurons. In weeks 26 to 28 a peak in transfer of afferent synaptic connections from the subplate to the cortical plate occurs.[1]

Proliferation of neurons continues to midgestation and maturation continues till the end of gestation.[2]

At 20 weeks most of the cortical surface is smooth and only the Sylvian fissure is present. From 20 weeks to 40 weeks gyration takes place with formation of primary, secondary and tertiary gyri and sulci. Brain volume increases linearly from 20 weeks gestation to term.[3] In this second half of gestation also synaptogenesis and dendritic arborization takes place and continues till after birth. The cortical plate is thickening in the third trimester. Axonal outgrowth to and from the cortex occurs during the second half of pregnancy as well and this process continues throughout infancy. Cortical layers are being formed between 24 to 34 weeks of gestation, each layer with its own specific type of neurons and connectivity.[1] The synaptogenesis starts earlier in the deeper layers than in more superficial layers, and is suggested to begin earlier in primary motor areas and later in anterior regions, such as the prefrontal cortex.

Microglia, oligodendrocytes, and astrocytes fulfill an important role in the development of white matter. These glial components support important functions for neurons in terms of supporting neuronal migration and modulation of synaptic connections. During gestation premyelinating oligodendrocytes are present in the developing cortex. Between 20 and 28 weeks of gestation myelin can be detected in the following order: first in the brainstem, capsula interna and after birth increasingly in the subcortical and cortical regions.[1] However, the majority of the white matter at 29 weeks of gestation is unmyelinated. After 36 weeks of gestation a rapid increase of myelinated white matter has occurred.[4] The formation of the myelin plays an important role in cerebral networking and control and modulation of the motor system.[2] As a consequence white matter injury has a major impact on long term neurodevelopmental outcome.

Cerebellar development starts early in gestation and continues during the first 20 months of life. A rapid growth of the cerebellum occurs between 24 to 40 weeks of gestation. During this period the cerebellar volume increases 5 fold, and foliation grows exponentially. This combination of events results in an 30-fold increase of cerebellar cortex surface.[5] Also neuronal migration in the cerebellum is active during the premature period.[6]

Considering the enormous growth and organization of neurons in the fetal brain, unexpected events during birth or in the neonatal period, both in preterm and term born infants, may have detrimental effects on cognitive and motor development later in life.

Term newborns

During the perinatal and postnatal period several risk factors for adverse events leading to brain injury can be identified. In term infants, one could think of peri- or postnatal infections, hypoglycemia, neonatal convulsions, perinatal asphyxia, persistent pulmonary hypertension due to meconium aspiration or other causes, and congenital abnormalities.

Perinatal asphyxia is one of the most important events leading to long term neurological sequelae in term newborns. During such events, oxygen delivery is compromised and thereby threatening the transition of the newborn at birth. Perinatal asphyxia occurs in 1–2/1000 deliveries in the Netherlands and is associated with a high risk on mortality or poor neurodevelopmental outcome. Premyelinating oligodendrocytes are particular sensitive to perinatal hypoxia or ischemia, which may disrupt myelination.[1] Studies have shown the effect of hypothermia after perinatal asphyxia. The effect of hypothermia is thought to influence secondary energy failure due to the release of cytokines and the production of free radicals, following the first perinatal hypoxic hit.[7] Since 2008, mild therapeutic hypothermia has been introduced in the Netherlands to prevent brain damage. Therapeutic hypothermia initiated within six hours after the primary hit in newborns with moderate to severe hypoxic-ischemic encephalopathy decreases the combined outcome of mortality and severe neurodevelopmental delay with a number needed to treat of seven.[8] Several studies have been performed, showing a better long term outcome for patients treated with therapeutic hypothermia at the age of 6 to 7 years.[9] However, only a few studies were performed in the Dutch population to investigate and confirm the short- and long term effects of therapeutic hypothermia in this population. Previous studies showed differences in neurodevelopmental outcome between the USA and the Dutch population using the Bayley Scales of Infant Development (BSID).[10]

Several causes can lead to perinatal asphyxia. One of these causes is acute anaemia due to umbilical cord rupture or vasa praevia, resulting in blood loss of the newborn. Acute anaemia leads to acute problems in gas exchange, resulting in respiratory and/ or circulatory disturbances. These respiratory and circulatory disturbances may lead to hypoxia and encephalopathy. Limited data is available on imaging, both ultrasound imaging and magnetic resonance imaging, and long term outcome in severe acute anaemic newborns.

Preterm newborns

Preterm birth itself is an important predictive risk factor for adverse neurodevelopmental outcome. Many other events during the neonatal period compromising development may occur. For example peri- or postnatal sepsis, necrotizing enterocolitis, metabolic problems like hypoglycemia, intracranial hemorrhage, and respiratory or circulatory insufficiency.

In preterm infants also the effect of adverse events leading to brain injury is slightly different, as these events occur during another stage of brain development in comparison to term infants. It is known that, due to the vulnerable vascularity in the preterm brain, these infants are at risk of intraventricular hemorrhage (IVH) and thus disturbing the migration of neurons from the germinal matrix.[11] The risk of IVH is inversely related to gestational age and birth weight.[12] IVH develops most frequently during the first week of life, with a peak incidence in the first three days of life. Infants with severe IVH clearly have an increased risk for neurodevelopmental outcome, especially if they develop post-hemorrhagic hydrocephalus.[13]

White matter injury also plays a prominent role in the development of adverse neurodevelopmental outcome. Preterm birth is associated with disruption of myelination.[1] Pre-oligodendrocytes predominate in the white matter throughout the high-risk period for white matter injury due to hypoxia-ischemia or inflammation.[14] This inflammation may already be present prenatally due to intra-uterine infection. But also postnatal infections, both early- as late-onset, have been identified as a major factor in the development of white matter injury.[15] Secondary to necrotic white matter injury, cortical and subcortical white matter may degenerate.[14] For decades now, it is known that infants with a cystic periventricular leukomalacia (PVL) have a poor prognosis.[16] Fortunately, the incidence of cystic PVL has reduced over the past decades, but has been replaced by more subtle white matter injury. In more recent studies more subtle damage in the white matter defined as punctate white matter lesions (PWML) has been evaluated. The extent of PWML and location of the lesions are associated with long term neurodevelopmental outcome.[17]

To detect brain abnormalities, diagnostics tools have been developed and can be used to predict long term outcome. The development of these diagnostic tools progresses quickly.

Cerebral Imaging

Cerebral ultrasonography has shown to be one of the most important diagnostic tools on the Neonatal Intensive Care Unit (NICU). Cerebral ultrasonography is one of the first imaging techniques used in neonatal care, and available bedside. It is an easy to use modality, which provides information on congenital or acquired brain injury, e.g. intraventricular hemorrhage, PVL of venous infarction.[18] Cerebellar sonography is emerging as standard of care in neonatal intensive care units. Increasing amounts of data become available about the effects of cerebellar hemorrhage on long term outcome.[19]

Imaging by ultrasonography improved over the years thanks to technological innovation, with increasing resolution of images. Studies have been published showing that changes in maturation of the deep gray matter can be detected over time.[20] Thereby, neuroimaging is of great clinical importance in daily decision-making. It can be used to identify and evaluate the development of cerebral abnormalities over time, especially when the condition of the patient is instable for transportation to undergo more advanced imaging techniques like Magnetic Resonance Imaging.

Magnetic Resonance Imaging (MRI) is an advanced technique in cerebral imaging. MRI provides more detailed and specific images of the young brain and possible lesions, however, it is not available bedside. It provides more detailed information about hemorrhage and white matter injury. Previous studies have shown more subtle white matter injury like PWML that cannot be detected with cerebral ultrasound.[17] This is important since this type of subtle damage has impact on long term outcome.

More advanced MRI techniques as diffusion tensor imaging and volumetry have been successfully used to improve prediction of long term outcome. Cerebral volume measurements and diffusor tensor imaging have been shown as promising techniques. Diffusion weighted imaging and spectroscopy are additionally valuable in term infants experiencing perinatal asphyxia,[21] to identify regions in the brain with diffusion restriction indicating post-ischemic changes and increased lactate levels.

Neurophysiological modalities: EEG and aEEG

Neurophysiological modalities are being used as well. Electroencephalography (EEG) can provide information about cortical activity, and the presence of convulsive activity.

Especially the background pattern of EEG and whether or not an anomalous background pattern recovers over time, can be of predictive value for long term neurodevelopmental outcome. A 21-channel EEG provides information of the entire brain. However, specialized knowledge is needed for a correct interpretation of the recordings. In addition, the duration of the registration is normally up to one hour and in certain cases a longer period of registration is desirable.

Brain activity and possible convulsive activity can be monitored for a longer period of time by using amplitude integrated EEG monitoring (aEEG). This aEEG is a two-channel EEG, with biparietal electrodes. The signal is filtered, compressed in time and is displayed in a semi-logarithmic scale. An aEEG provides background patterns, which can be interpreted by pattern recognition, and is therefore very usable as a bedside technique. These background patterns can help to predict neurological prognosis.[22, 23] In term infants the use of an EEG is widespread. In the past it was less frequently used in preterm infants, but nowadays more and more data become available, showing the applicability in this younger group.[24, 25]

Other modalities: Near Infrared Spectroscopy

Another modality to monitor neurophysiological parameters is Near Infrared Spectroscopy (NIRS). This bedside technique provides information about regional cerebral oxygen saturation ($rScO_2$). This modality can provide an additional parameter in monitoring, e.g. by detecting changes in cerebral perfusion, which may prelude cerebral damage. It is suggested that cerebral hyperperfusion might procede the development of severe peri-intraventricular haemorrhage.[26] Reference values have been published for preterm infants in the first three days of life.[27] NIRS is being used more extensively, also to monitor splanchnic oxygenation.[28] It could be an auspicious modality in clinical monitoring with little impact on the newborn. Although NIRS monitoring is often used as part standard of care, the role of these results in clinical decision-making is still under debate.[29]

Neurodevelopmental outcome

As mentioned before, long term neurodevelopmental outcome is an important outcome parameter of neonatal intensive care. Several measurement tools are used to quantify this long term outcome.

In the first year of life the focus of neurodevelopment is mainly on gross motor development. One of the tools used to quantify gross motor development is the Alberta Infant Motor Scale (AIMS). The AIMS is designed to measure motor skills from term equivalent age to independent walking, sit and stand.[30] Components tested in the AIMS are weight bearing properties, posture and antigravity movements.[31] AIMS is not designed as a predictive tool, but it has moderate to excellent predictive validity. It has been used to classify the infants development from as normal or suspicious/ abnormal at twelve months corrected age.[32]

Another tool for assessment of long term neurodevelopmental outcome is the Bayley Scales of Infant and Toddler Development (BSID). In the Dutch Neonatal Follow Up program for preterm infants the BSID is used to assess the neurodevelopment at the corrected age of two years. Both mental (mental developmental index, MDI) and psychomotor development (psychomotor developmental index, PDI) are assessed. The BSID is generally used to assess neurodevelopmental outcome in preterm born infants. During the past years, BSID has been improved and newer versions of BSID are available. The BSID second version has a poor predictive value for cognitive outcome at the age of eight years, showing that children with severe handicaps and/ or subnormal functioning at the age of two years had improved at the age of eight years.[33] On the other hand, the children with a score in the subnormal range had poorer school age functioning compared to children with a score in the normal range,[33] thus this tool can be used to identify the vulnerable children at preschool age.[34] The current version, BSID-III, has an improved predictive value compared to BSID-II, with a strong predictive validity to predict normal and abnormal cognitive outcomes in preterm infants.[35] However, the predictive value of BSID for later development is still limited.[36] Therefore, it would be preferable to assess the prognoses for infants admitted at a Neonatal Intensive Care Unit on a combination of prognostic tools.

Aim and outline of this thesis

Long term neurodevelopmental outcome is the main result of a neonatal intensive care period. Factors influencing this outcome are of utmost importance. It is therefore needed to identify these factors, which in the end can lead to preventive measures.

Therefore, the aim of this thesis is assessment of specific predictive factors during perinatal and neonatal period, leading to brain damage and subsequently to deterioration of long term neurodevelopmental outcome. In the term born infant we focus on perinatal events, and in the preterm infant postnatal infection was investigated.

Part 1: Perinatal predictive factors

In part 1 perinatal predictive factors for adverse neurodevelopmental outcome are described. Chapter 2 addresses acute anaemia in the perinatal period. If acute anaemia occurs during birth, cerebral oxygen supply may be impaired. Studies have described short term outcome, however, in literature long term outcome data are scarce. Cerebral MRI imaging following the perinatal event may clarify whether brain injury has occurred. It is questioned whether MRI imaging could help to predict long term outcome in these infants.

In chapter 3 we focus on the effect of therapeutic hypothermia in newborns with perinatal asphyxia. In the Netherlands in 2008 and 2009 therapeutic hypothermia has been introduced in all NICU's. In previous international studies hypothermia has proven to have positive effects on long term outcome. In this study we investigate the outcome of hypothermia in a Dutch population.

Part 2: Postnatal risk factors: Infection

In part 2 the effect of late-onset infection in preterm infants is evaluated.

Chapter 4 discusses postnatal imaging performed in preterm infants suspected of late-onset blood stream infection. We investigate whether abnormalities can be detected using cerebral imaging during or after late-onset sepsis in comparison to preterm infants without late-onset sepsis.

Chapter 5 addresses the use of NIRS for cerebral monitoring in the same population. With Near Infrared Spectroscopy we aim to study the effect of late-onset sepsis on cerebral oxygenation in the preterm infant.

Chapters 6 and 7 address the long term neurodevelopmental outcome in preterm infants experiencing late-onset sepsis. In chapter 6 early motor outcome at the age of six and twelve months corrected age, using the AIMS is studied. Chapter 7 covers the neurodevelopmental outcome at two years corrected age, using the BSID.

References

1. Tau GZ, Peterson BS. Normal development of brain circuits. Neuropsychopharmacology. 2010;35(1):147-68.
2. Adams-Chapman I. Insults to the developing brain and impact on neurodevelopmental outcome. J Commun Disord. 2009;42(4):256-62.
3. Kinney HC. The near-term (late preterm) human brain and risk for periventricular leukomalacia: a review. Semin Perinatol. 2006;30(2):81-8.
4. Hüppi PS, Warfield S, Kikinis R, et al. Quantitive Magnetic Resonance Imaging of Brain Development in Premature and Mature Newborns. Annals of Neurology. 1998;43:224-35.
5. Volpe JJ. Cerebellum of the Premature Infant: Rapidly Developing, Vulnerable, Clinically Important. J Child Neurol. 2009;24:1085-104.
6. Volpe JJ. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. The Lancet Neurology. 2009;8(1):110-24.

7. Peeters C, van Bel F. Pharmatherapeutical Reduction of Post-Hypoxic-Ischemic Brain Injury in the Newborn. Biol Neonate. 2001;79:274-80.
8. Jacobs SE, Berg M, Hunt R, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev. 2013(1):CD003311.
9. Azzopardi D, Strohm B, Marlow N, et al. Effects of hypothermia for perinatal asphyxia on childhood outcomes. N Engl J Med. 2014;371(2):140-9.
10. Westera JJ, Houtzager BA, Overdiek B, et al. Applying Dutch and US versions of the BSID-II in Dutch children born preterm leads to different outcomes. Dev Med Child Neurol. 2008;50(6): 445-9.
11. Wyatt JS. Mechanisms of brain injury in the newborn. Eye (Lond). 2007;21(10):1261-3.
12. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal Outcomes of Extremely Preterm Infants From the NICHD Neonatal Research Network. Pediatrics. 2010;126:443-56.
13. Adams-Chapman I, Hansen NI, Stoll BJ, et al. Neurodevelopmental outcome of extremely low birth weight infants with posthemorrhagic hydrocephalus requiring shunt insertion. Pediatrics. 2008;121(5):e1167-77.
14. Back SA. Brain Injury in the Preterm Infant: New Horizons for Pathogenesis and Prevention. Pediatr Neurol. 2015;53(3):185-92.
15. Adams-Chapman I. Long-term impact of infection on the preterm neonate. Semin Perinatol. 2012;36(6):462-70.
16. Perlman J. White matter injury in the preterm infant: an important determination of abnormal neurodevelopmental outcome. Early Human Development. 1998;53(2):99-120.
17. Tusor N, Benders MJ, Counsell SJ, et al. Punctate White Matter Lesions Associated With Altered Brain Development And Adverse Motor Outcome In Preterm Infants. Sci Rep. 2017;7(1):13250.
18. Maller VV, Cohen HL. Neurosonography: Assessing the Premature Infant. Pediatr Radiol. 2017;47(9):1031-45.
19. Steggerda SJ, De Bruine FT, van den Berg-Huysmans AA, et al. Small cerebellar hemorrhage in preterm infants: perinatal and postnatal factors and outcome. Cerebellum. 2013;12(6):794-801.
20. de Goederen R, Raets MMA, Ecury-Goossen GM, et al. Effect of Preterm Birth on Echogenicity in Basal Ganglia. Ultrasound Med Biol. 2017;43(10):2192-9.
21. Alderliesten T, de Vries LS, Benders M, et al. MR Imaging and Outcome of Term Neonates with Perinatal Asphyxia: Value of Diffusion-weighted MR Imaging and 1H MR Spectroscopy. Radiology. 2011;261(1):235-42.
22. Toet M, Hellstrom-Westas L, Groenendaal F, et al. Amplitude integrated EEG 3 en 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed. 1999;81:F19-F23.
23. Hellström-Westas L, Rosén I, Svenningsen NW. Predictive value of early continuous amplitude integrated EEG recordings on utcome after severe birth asphyxia in full term infants. Arch Dis Child. 1995;72:F34-F8.
24. Huning B, Storbeck T, Bruns N, et al. Relationship between brain function (aEEG) and brain structure (MRI) and their predictive value for neurodevelopmental outcome of preterm infants. Eur J Pediatr. 2018;177(8):1181-9.
25. Middel RG, Brandenbarg N, Van Braeckel K, et al. The Predictive Value of Amplitude-Integrated Electroencephalography in Preterm Infants for IQ and Other Neuropsychological Outcomes at Early School Age. Neonatology. 2018;113(4):287-95.
26. Alderliesten T, Lemmers PM, Smarius JJ, et al. Cerebral oxygenation, extraction, and autoregulation in very preterm infants who develop peri-intraventricular hemorrhage. J Pediatr. 2013;162(4):698-704 e2.
27. Alderliesten T, Dix L, Baerts W, et al. Reference values of regional cerebral oxygen saturation during the first 3 days of life in preterm neonates. Pediatr Res. 2016;79(1-1):55-64.

28. Schat TE, Schurink M, van der Laan ME, et al. Near-Infrared Spectroscopy to Predict the Course of Necrotizing Enterocolitis. PLoS One. 2016;11(5):e0154710.
29. Kooi EMW, Verhagen EA, Elting JWJ, et al. Measuring cerebrovascular autoregulation in preterm infants using near-infrared spectroscopy: an overview of the literature. Expert Rev Neurother. 2017;17(8):801-18.
30. Darrah J, Piper M, Watt MJ. Assessment of gross motor skills of at-risk infants: predictive validity of the Alberta Infant Motor Scale. Dev Med Child Neurol. 1998;40(7):485-91.
31. Spittle AJ, Doyle LW, Boyd RN. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. Dev Med Child Neurol. 2008; 50(4):254-66.
32. George JM, Boyd RN, Colditz PB, et al. PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome. BMC Pediatr. 2015;15:123.
33. Hack M, Taylor HG, Drotar D, et al. Poor predictive validity of the Bayley Scales of Infant Development for cognitive function of extremely low birth weight children at school age. Pediatrics. 2005;116(2):333-41.
34. Roberts G, Anderson PJ, Doyle LW, et al. The stability of the diagnosis of developmental disability between ages 2 and 8 in a geographic cohort of very preterm children born in 1997. Arch Dis Child. 2010;95(10):786-90.
35. Bode M, D'Eugenio DB, Mettelman BB, et al. Predictive Validity of the Bayley, Third Edition at 2 Years fot Intelligence Quotient at 4 Years in Preterm Infants. J Dev Behav Pediatr. 2014;35:570-5.
36. Luttikhuizen dos Santos ES, de Kieviet JF, Konigs M, et al. Predictive value of the Bayley scales of infant development on development of very preterm/very low birth weight children: a meta-analysis. Early Hum Dev. 2013;89(7):487-96.

Part 1

Perinatal predictors

Chapter 2

Severe neonatal anaemia, MRI findings and neurodevelopmental outcome

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Abstract

Background and objective:

Severe neonatal anaemia can impair cerebral oxygen supply. Data on long-term outcomes following severe neonatal anaemia are scarce.

Methods:

Clinical data and neurodevelopmental outcome of 49 (near) term infants with haemoglobin concentration after birth <6.0 mmol/L were retrospectively collected and analysed. In a subgroup of 28 patients amplitude integrated aEEG was available and in 25 infants cerebral MRI was obtained. Infants were followed-up at 14–35 months of age and assessed with the Griffiths Scale of Mental Development or Bayley Scales of Infant Development.

Results:

Eighteen patients (37%) deceased during the neonatal period. In 25 patients MRI was performed. A predominant pattern of injury on MRI was seen in basal ganglia and thalami in seven patients (28%), whereas any grade of white matter injury was present in 16 (64%) and a combination in 3 (12%). Follow-up data were available for 26 patients (84% of survivors). Formal assessment of neurodevelopmental outcome was performed in 20 of 31 (65%) infants who survived (median age 19 months, range 14–35 months). Sixteen infants (80%) had a developmental quotient appropriate for age in the first two years after birth. On motor outcome one patient (5%) scored below average (Z-score -1.10). One patient developed cerebral palsy.

Conclusion:

Early neurodevelopmental outcome in surviving patients with severe neonatal anaemia was within the normal range in the majority of the survivors. MR imaging showed mild to moderate white matter injury in two thirds of infants. Prospectively collected data with a longer follow-up period are needed.

Introduction

Neonatal anaemia has a diverse aetiology, and it can cause an acute life threatening situation through hypovolaemic shock and hypoxia at birth, as well as through multiple organ failure in the first days of life. During hypovolaemic shock redistribution of blood flow occurs and may be preferentially directed to the brain which possibly prevents more severe adverse neurological sequelae.

Anaemia may result in encephalopathy, which can be assessed using aEEG (amplitude integrated EEG).

Data on MRI findings and neurodevelopmental outcome of survivors of neonatal anaemia are limited. Since severe anaemia is often associated with perinatal asphyxia, it is hard to distinguish with neuro-imaging whether cerebral injury is due to anaemia or the associated hypoxia-ischaemia.

The aim of this retrospective study is to describe aEEG and MRI findings following severe neonatal anaemia in (near) term infants admitted at a level III Neonatal Intensive Care Unit, and relate these findings to neurodevelopmental outcome at approximately two years of age.

Methods

We retrospectively collected clinical data from medical records of patients who presented with severe anaemia in the NICU of the VU Medical Center, Amsterdam, or the Wilhelmina Children's Hospital, University Medical Center, Utrecht in the period January 2000 to June 2011.

Patients with an initial haemoglobin (Hb) concentration <6.0 mmol/L ([mg/dL] = [mmol/L] x 1.61) and a gestational age ≥36 weeks were eligible for this study. Infants with chromosomal abnormalities or inborn errors of metabolism were excluded.

In nine patients, the first Hb-concentration was measured after a blood transfusion had already been administered immediately after birth. Initial Hb-concentrations were therefore estimated, based on the fact that a blood transfusion of 20 ml/kg increases Hb-concentration with approximately 3.0 mmol/L. These estimated values were used as the initial Hb-concentrations.

Various clinical data of vital organ failure were collected, including need for respiratory or circulatory support and the aEEG background pattern.

MRI analysis

Magnetic resonance imaging (MRI) in the VU Medical Center was performed on a 1.5 Tesla magnet (Siemens Vision, Erlangen, Germany). The MRI protocol included T1, T2 and diffusion weighted images (DWI). In the Wilhelmina Children's Hospital the same sequences were obtained on a 1.5 Tesla MRI, and more recently on a 3 Tesla MRI (respectively ACS-NT system and Achieva, Philips Medical Systems, Best, the Netherlands)

We assessed predefined brain areas on both conventional imaging and DWI. The MRI score described by Rutherford was used (*Appendix 2.1*). The separate areas of interest were scored in three categories: 1) no abnormalities (no abnormalities on T1 and T2 weighted images), 2) mild abnormalities (focal regions of abnormal signal intensity) and 3) moderate to severe abnormalities (multiple regions of abnormal signal intensity).

Outcome

Neurodevelopmental and neuromotor function follow-up were assessed with either the Griffiths Scale of Mental Development (GMDS), Bayley Scales of Infant and Toddler Development-second edition-Dutch version (BSID-II-NL) or, more recently, Bayley Scales of Infant and Toddler Development third edition (BSID-III). In order to enable the comparison of these different developmental tests, Z-scores were calculated for GMDS (Performance Developmental Quotient (PDQ) used for neurodevelopment and the Locomotor Developmental Quotient (LDQ) used for motor development), BSID-II-NL (Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI)), and BSID-III composite score outcomes. Z-scores were calculated as follows: [(score - test mean) / standard deviation (SD)] for each test. Development was classified as either mildly delayed (Z-score $\leq$-1), or normal (Z-score >-1). If neurodevelopmental tests were not performed, parents and/or general practitioners were contacted by phone to obtain the most recent status of neurodevelopment.

Statistical analysis

Comparisons were made between groups with an initial Hb concentrations $\leq$3.0 mmol/L and 3.1–6.0 mmol/L, as well as between surviving and deceased patients. Comparisons were also made between groups with and without MRI abnormalities. We used the Chi square test and the Mann Whitney U test for categorical and continuous variables respectively. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using SPSS Software Package version 20.

Results

Patient characteristics are presented in *Table 2.1*. Hb-concentrations were measured at birth or within several hours after birth and ranged from 1.0 mmol/L to 5.9 mmol/L.

Table 2.1: Patient characteristics

Clinical characteristics (n=49)	N (%) or median (range)
Gestational age (weeks)	39 4/7 (36 0/7–42 1/7)
Birth weight (grams)	3200 (1850–4305)
Sex (male)	26 (53%)
Apgar score at 5 min	4 (0–10)
pH (umbical cord or first pH)	6.96 (6.60–7.30)
Lactate max	19.3 (2.9–35.0)
Haemoglobin concentration (mmol/L)	4.1 (1.0–5.9)
Survival	31 (63.3%)
aEEG performed	42 (86%)
aEEG background pattern documented	28 (57%)
MRI performed	25 (51%)
Survivors with MRI imaging	20 (40.8%)
Neurodevelopmental assessment of survivors (n=31)	20 (64.5%)
Neurodevelopmental assessment and MRI	14 (45.2%)

N, number; BE, base excess; aEEG, amplitude integrated EEG.

Aetiology of neonatal anaemia

The most frequent causes of anaemia were foetomaternal haemorrhage (30.6%) and exsanguination (26.5%) due to vasa or placenta praevia. Less common causes were twin-to-twin transfusion syndrome (8.2%), placental abruption (8.2%), bleeding due to a traumatic delivery (8.2%), umbilical cord rupture (6.1%), haemolysis (6.1%) and complications during caesarean section (2.0%). In 4.1% the aetiology remained unknown. As hydrops was not present in any patient, the onset of the severe anaemia was most likely to be (sub)acute. Reticulocyte count data would have been useful to help determine the time of onset of the anaemia, but these data were not available in most of the infants.

Clinical characteristics

Invasive mechanical ventilation was required in 85.7%, and non-invasive respiratory support (CPAP: Continuous Positive Airway Pressure) in 14.3%. Circulatory support with vasopressors was needed in 75.5% of the patients. There were no significant differences in the need of support of vital organs or mortality between the two Hb-concentration groups (*Table 2.2*).

Thirteen patients (31%) had at least one glucose concentration <2.0 mmol/L. There were no differences in mortality (Fisher exact test, p=0.759), morbidity (Fisher exact test, need for respiratory support p=0.186, need for circulatory support p=0.636, convulsions (either clinical or electrographic) p=0.612) or neurodevelopment or motor development (Chi square test, resp. p=0.954 and p=0.683) between normoglycaemic and hypoglycaemic patients.

Table 2.2: Clinical characteristics of survivors vs deceased and haemoglobin concentration below or above 3.0 mmol/L

	Survivors n=31	Deceased n=18	p-value	Hb <3.0 mmol/L n=14	Hb 3.1–6.0 mmol/L n=35	p-value
Invasive resp. support	24 (77%)	18 (100%)	0.030	14 (100%)	28 (80%)	0.073
Vasopressors	19 (61%)	18 (100%)	0.002	11 (79%)	26 (74%)	0.759
Glucose <2.0 mmol/L	9 (29%)	6 (33%)	0.759	6 (43%)	9 (26%)	0.309
aEEG pattern BS/FT	4/19 (21%)	7/9 (89%)	0.003	1/6 (17%)	10/22 (45%)	0.215
MRI performed	20 (65)%	5 (28%)	0.013	6 (43%)	19 (54%)	0.480
Hb <3.0 mmol	9 (29%)	5 (28%)	0.927			
Survival				9 (64%)	22 (63%)	0.927

Hb, Haemoglobin; aEEG, amplitude integrated electroencephalogram; BS, Burst suppression; FT, Flat Trace, Chi square test.

aEEG characteristics

Clinical or subclinical seizures were noted in 61.2% of the infants (n=30). Of these, 43.3% of the infants (n=13) needed one, and 56.6% (n=17) needed multiple anti-epileptic drugs (AED). Most patients were monitored with aEEG during the first days after birth (n=42, 86%). In 28 patients the aEEG was available for assessment of the background pattern. The patterns recorded and scored during the first 12 hours after birth were used. Continuous Normal Voltage was found in 50% (n=14), Discontinuous Normal Voltage in 7.1% (n=2), Burst Suppression in 32.1% (n=9), Continuous Low Voltage in 3.6% (n=1), and Flat Trace in 7.1% (n=2) of the infants. No differences in seizure activity (Fisher exact test, p=0.754) or aEEG background pattern (Fisher exact test, p=0.355) were observed for the two Hb-concentration groups. The aEEG background patterns in the deceased group were more often non-favourable (Burst Suppression or Flat Trace) (Fisher exact test, p=0.010). These non-favourable patterns were associated with motor developmental delay in the surviving patients (Chi square test, p=0.001). For neurodevelopment, non-favourable patterns showed a trend towards delay (Chi square test, p=0.100).

Neonatal outcome

Eighteen patients (36.7%) did not survive the neonatal period. Thirteen patients died within 72 hours after birth, mainly due to acute multiple organ failure. The other five patients died following a decision to redirect care due to the expected serious long-term sequelae, based on clinical parameters (e.g. therapy resistant convulsions) and/or extensive cerebral damage documented by MRI. The deceased patients had a significantly higher need for respiratory and circulatory support compared to the living patients, but there was no difference in the presence of seizure activity.

MRI analysis

Imaging was performed in 25 patients, between day 2 and 8 after birth (median: day 6). Five of the patients died, in three patients MRI imaging was performed post-mortem. DWI images were available of 18 infants imaged during life. The clinical characteristics of infants with an MRI were not significantly different compared to the total study population.

In three deceased patients MRI imaging was obtained post-mortem. In one deceased patient DWI (MRI obtained during life on day 6) showed few abnormalities in contrast to the conventional images that were clearly abnormal, possibly due to antenatal onset of the anaemia.

White matter injury

In 16 of the 25 patients (64%) any grade of white matter injury was found, both in surviving and deceased patients. Severe widespread abnormalities were seen in eight infants (32%), three of whom died. Another eight infants (32%) had mild white matter lesions.

Severe lesions were seen in the occipital white matter (n=8) and slightly less often in the frontal (n=6) and temporal white matter (n=7). Mild lesions were seen in occipital (n=4), frontal (n=6) and temporal white matter (n=1). White matter lesions were often present in more than one site (*Table 2.3*).

No relation was found between white matter abnormalities and an unfavourable aEEG background pattern or the presence of hypoglycaemia (n=28, Fisher exact test, p=0.315

Table 2.3: MRI findings in the total population

	Conventional images (n=25)		Diffusion weighted images (n=19)
	Severe abnormalities	Mild abnormalities	Abnormalities
Occipital WM	8 (32%)	4 (16%)	5 (26%)
Frontal WM	6 (24%)	6 (24%)	4 (21%)
Temporal WM	7 (28%)	1 (4%)	3 (16%)
All WM	16 (64%)		6 (32%)
WM injury more than 1 site	7 (28%)	2 (8%)	4 (21%)
Basal ganglia and thalami	5 (20%)	3 (12%)	3 (16%)
Internal capsule	6 (24%)	1 (4%)	3 (16%)

WM, white matter.

and p=0.205 respectively). There were also no differences in Locomotor Developmental Quotient/ Psychomotor Developmental Index (LDQ/ PDI), Performance Developmental Quotient/ Mental Developmental Index (PDQ/ MDI) or combined outcome death or motor/ neurodevelopmental outcome Z-score <-1 (n=14, Chi square test, p=0.901, p=0.902 and p=0.154 respectively).

Basal ganglia/thalamic (BGT) injury

Abnormalities in the basal ganglia and thalami were seen in seven infants. Moderate to severe abnormalities were only seen in five deceased infants, all of whom had associated white matter lesions. Mild BGT abnormalities were found in two (8%) surviving patients, without associated white matter lesions.

Comparison of infants with Hb <3 mmol/L and 3.1–5.9 mmol/L

Severity of anaemia did not show a relation with subsequent brain abnormalities. This is illustrated in *Figure 2.1*. There was no difference in mortality between the two Hb-concentration groups.

Cerebellar damage was seen in six infants, in both Hb-concentration groups (*Table 2.4*). Haemorrhagic lesions were found in three infants, while ischaemic lesions were found in the other three infants.

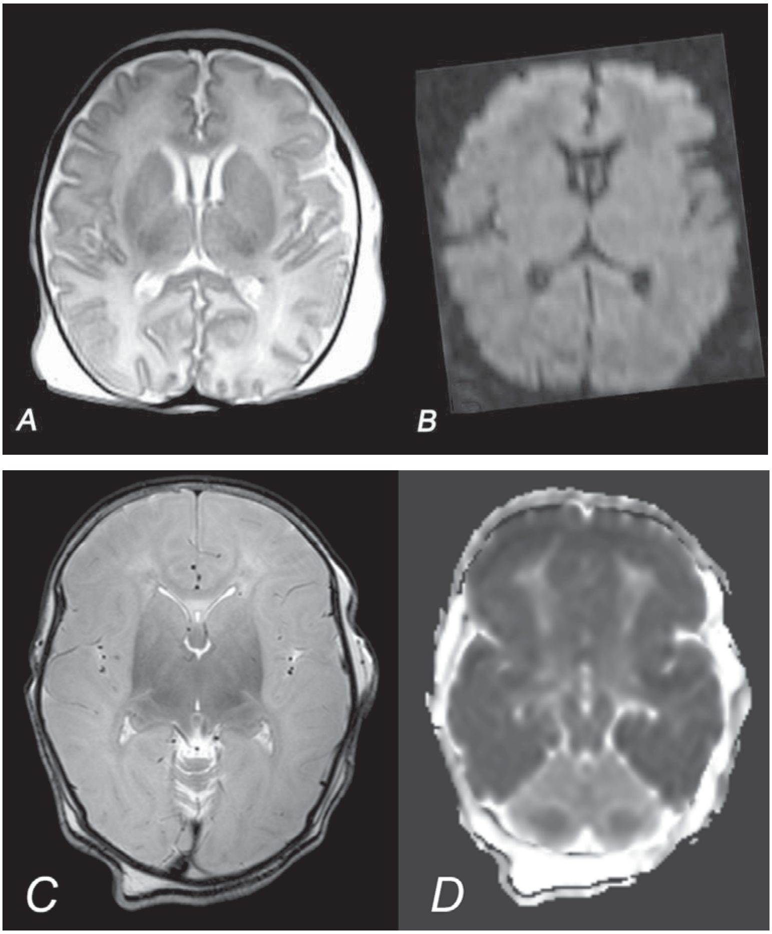

Figure 2.1: MRI imaging.
A and B, MRI day 7: Term born male with Apgar scores 5 and 6 at 1 and 5 min and an initial Hb-concentration of 1.9 mmol/L (A: axial T2 weighted image at the level of the basal nuclei, B: axial DWI): mild abnormalities in occipital white matter, not seen as increased signal intensity on DWI. Patient did not appear on follow-up appointments, but had a favourable outcome according to the general practitioner at the age of two years. C and D: Term born female, Apgar scores 4 and 8 at 1 and 5 min and an initial Hb-concentration of 5.6 mmol/L, MRI day 2 (C: axial T2 weighted image, D: axial ADC map). Diffuse severe abnormalities throughout the cortex and white matter, near total brain injury (ADC thalami 700 x 10^{-6} mm^2/s, basal ganglia 850 x 10^{-6} mm^2/s. Care was redirected.

Neurodevelopmental outcome

Neurodevelopmental test results were available in 20 of the 31 surviving patients (median age 19 months, range 14–35 months). Mean Z-score for neurodevelopment was 0.43 (SD 0.71). Only one patient (5%) was mildly delayed (Z-score -1.08). Mean Z-score for motor outcome was 0.58 (SD 0.81). One patient (5%) was mildly delayed (Z-score -1.10).

Table 2.4: Abnormal MRI findings (conventional images) of survivors vs deceased and Hb concentration below or above 3.0 mmol/L

	Survivors n=20	Deceased n=5	p-value	Hb <3.0 mmol/L n=6	Hb 3.1–6.0 mmol/L n=19	p-value
Occipital WM	7 (35%)	5 (100%)	0.035	5 (83%)	5 (26%)	0.121
Temporal WM	4 (20%)	4 (80%)	0.012	3 (50%)	5 (26%)	0.514
Frontal WM	7 (35%)	5 (100%)	0.060	3 (50%)	9 (47%)	0.926
BGT	3 (15%)	5 (100%)	0.001	1 (17%)	7 (37%)	0.624
Internal capsule	2 (10%)	5 (100%)	<0.001	1 (17%)	6 (32%)	0.642
Cerebellum	1 (5%)	5 (100%)	<0.001	1 (17%)	5 (26%)	0.733

WM, White matter; BGT, basal ganglia and thalami. Fisher exact test.

Unilateral spastic cerebral palsy was diagnosed in one patient with haemorrhagic cortical infarction. Of the 11 patients who were not formally tested, information was retrieved from the paediatrician, general practitioner or the parents. In none of these patients cerebral palsy was diagnosed and in one patient behavioural problems were reported. Information about outcome could not be retrieved in two patients.

Thirteen of the 20 survivors with available MRI data were tested. Median age of testing was 24 months (range 15–35 months). The mean Z-score for neurodevelopment was 0.37 (SD 0.76) and the mean Z-score for motor outcome was 0.67 (SD 0.88). No differences were found in neurodevelopmental and motor outcome between patients with no/mild white matter abnormalities and patients with moderate to severe white matter abnormalities.

There were no differences between the two Hb-concentration groups for the neurodevelopmental outcome (mean Z-score resp. 0.33 (SD 0.79) and 0.73 (SD 0.59)) and the motor outcome (mean Z-score resp. 0.62 (SD 0.88) and 0.49 (SD 0.62)).

Discussion

In this retrospective study we were able to show that lesions in the BGT and especially in the white matter are a common finding in infants with severe neonatal anaemia.

Cerebral damage has been suggested to be caused through several pathophysiological mechanisms. In full-term infants, the deep grey matter nuclei are probably affected after an acute hypoxic-ischaemic insult due to changes in brain maturation and increased metabolic demands.[1] White matter injury is also found in full-term infants and is considered to be due to more prolonged and repetitive hypoxic-ischaemic events. A combination of deep grey matter damage and white matter lesions can also be found.[2] We hypothesize that the mechanisms that cause basal ganglia and white matter injury following severe anaemia might be similar to the mechanisms responsible for cerebral injury in full-term infants with perinatal asphyxia due to other causes. White matter injury has been described in animals as well as in humans after moderate prolonged foetal or neonatal asphyxia, as well as after neonatal hypoglycaemia.[3, 4] It is important to note that a substantial percentage (31%) of our anaemic patients also had a period of hypoglycaemia. The mechanism of injury due to hypoglycaemia is still unclear, although it has been hypothesized that it is caused by an increased regional cerebral blood flow during hypoglycaemia with a subsequent reduction in regional glucose uptake.[3]

Even more interesting are the clinical implications of these findings. MRI is a well established method to assess brain injury in infants suffering from perinatal asphyxia, and these findings are closely related to later neurodevelopmental outcome.[4-10] Data on the long-term outcome of patients with severe acute anaemia combined with a less severe component of perinatal asphyxia are still limited. It is of interest that early neurodevelopmental and motor outcomes in the first two years of life in the survivors is favourable. In the absence of damage in the thalamus and basal ganglia neonatal anaemia itself does not have a significant effect on outcome at two years of age.[11] However, the children are still young and their outcome should be re-assessed at school age. We also found that the severity of anaemia does not influence the need for intensive care treatment in the neonatal period. Moreover, we also found that there was no significant difference in survival between patients with an initial Hb-concentration ≤3.0 mmol/L versus patients with an initial Hb-concentration between 3.1–6.0 mmol/L. There was also no increased need for respiratory or circulatory support in the infants with more severe anaemia. However, the degree of organ failure and especially the degree of encephalopathy scored clinically or with aEEG, the presence of seizure activity and of severe injury on MRI were correlated with mortality and/or redirection of care.

Limitations

This retrospective study has several limitations. First, Hb-concentrations were not assessed within a predefined time after birth. However, all Hb measurements were performed within 6 hours after birth, indicating severe neonatal anaemia. Second, MRI imaging was initially restricted to infants with a clinical indication, although MRI was also performed in patient with anaemia without serious co-morbidity. Although this may have caused a potential bias, clinical characteristics in infants with and without MRI data were not different. Third, a full neurodevelopmental assessment was not performed routinely. It would therefore be desirable to have a follow-up in these patients at school age to study whether the white matter lesions are associated with school performance, as has previously been shown in survivors with white matter injury in the context of hypoxic ischaemic encephalopathy.[12] More insight in the association between patterns of damage and possible pathophysiological mechanisms, combined with neurological outcome, will hopefully provide more insight in how to predict future disabilities during the newborn period.

In conclusion

Severe neonatal anaemia is associated with high neonatal mortality and neonatal morbidity. Those who survive perform relatively well when assessed at approximately two years of age.

MR imaging shows abnormalities in the basal ganglia and thalami in severely affected infants and white matter lesions in most patients. However, it is difficult to distinguish between damage due to anaemia only and damage due to associated perinatal asphyxia.

Due to the retrospective setup of this study and missing data, results should be interpreted with caution. Prospectively collected data with a longer follow-up period are needed.

Appendix 2.1

MRI scoring system[10]

Posterior limb of internal capsule (PLIC): 0 = normal, 1 = reduced or asymmetrical signal intensity; 2 = severe injury with reversed or abnormal signal intensity bilaterally on T1 and or T2 weighted images.

Basal ganglia and thalami (BGT): 0 = normal, 1 = mild injury (focal abnormal signal intensity); 2 = moderate injury (multifocal abnormal signal intensity); 3 = indicates severe injury (widespread abnormal signal intensity).

White matter (WM): 0 = normal; 1 = mild injury (long T1 and T2 in periventricular white matter only); 2 = long T1 and T2 in subcortical WM and or focal punctate lesions or focal infarction; 3 = severe widespread abnormalities including long T1 and T2, infarction and haemorrhage.

Cortex: 0 = normal; 1 = mild (1–2 sites cortical highlighting / decreased T1); 2 = moderate (3 sites involved); 3 = severe (more than three sites).

References

1. Wyatt JS. Mechanisms of brain injury in the newborn. Eye (Lond). 2007;21(10):1261-3.
2. Okereafor A, Allsop J, Counsell SJ, et al. Patterns of brain injury in neonates exposed to perinatal sentinel events. Pediatrics. 2008;121(5):906-14.
3. Burns CM, Rutherford MA, Boardman JP, et al. Patterns of cerebral injury and neurodevelopmental outcomes after symptomatic neonatal hypoglycemia. Pediatrics. 2008;122(1):65-74.
4. Martinez-Biarge M, Bregant T, Wusthoff CJ, et al. White matter and cortical injury in hypoxic-ischemic encephalopathy: antecedent factors and 2-year outcome. J Pediatr. 2012;161(5):799-807.
5. Anderliesten TV, de Vries LS, Benders M, et al. MR Imaging and Outcome of Term Neonates with Perinatal Asphyxia: Value of Diffusion-weighted MR Imaging and 1H MR Spectroscopy. Radiology. 2011;261(1):235-42.
6. Lally PJ, Price DL, Pauliah SS, et al. Neonatal encephalopathic cerebral injury in South India assessed by perinatal magnetic resonance biomarkers and early childhood neurodevelopmental outcome. PLoS One. 2014;9(2):e87874.
7. Martinez-Biarge M, Diez-Sebastian J, Kapellou O, et al. Predicting motor outcome and death in term hypoxic-ischemic encephalopathy. Neurology. 2011;76:2055-61.
8. Miller SP, Ferriero DM, Leonard C, et al. Early brain injury in premature newborns detected with magnetic resonance imaging is associated with adverse early neurodevelopmental outcome. J Pediatr. 2005;147(5):609-16.
9. Rutherford M, Ramenghi LA, Edwards AD, et al. Assessment of brain tissue injury after moderate hypothermia in neonates with hypoxic-ischaemic encephalopathy: a nested substudy of a randomised controlled trial. Lancet Neurol. 2010;9:39-45.
10. Vermeulen RJ, van Schie PEM, Hendrikx L, et al. Diffusion-weighted and conventional MR Imaging in Neonatal Hypoxic Ischemia: Two-year Follow-up Study. Radiology. 2008;249(2):631-9.
11. Harteman JC, Groenendaal F, Toet MC, et al. Diffusion-weighted imaging changes in cerebral watershed distribution following neonatal encephalopathy are not invariably associated with an adverse outcome. Dev Med Child Neurol. 2013;55(7):642-53.
12. Perez A, Ritter S, Brotschi B, et al. Long-term neurodevelopmental outcome with hypoxic-ischemic encephalopathy. J Pediatr. 2013;163(2):454-9.

Chapter 3

Comparison by questionnaires of psychomotor outcome in patients with perinatal asphyxia with versus without therapeutic hypothermia at 4 years

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Abstract

Background and objective:

Therapeutic hypothermia improves outcome after perinatal asphyxia. The Ages and Stages Questionnaire is a screening tool to detect neurodevelopmental delay. In this study we examined the outcome of patients with perinatal asphyxia (defined as Apgar score <5 at 10 minutes, or continued need for resuscitation, or pH <7.00 in umbilical cord or within one hour after birth) with and without therapeutic hypothermia treatment at the age of four years.

Methods:

Cohort study of patients with perinatal asphyxia admitted to the Neonatal Intensive Care Units of the VU University Medical Center, Amsterdam and the Wilhelmina Children's Hospital, Utrecht in the year 2008. Parents were asked to fill out the 48 months Ages and Stages Questionnaire (ASQ). In Wilhelmina Children's Hospital treatment with therapeutic hypothermia was implemented in 2008, in the VU University Medical Center in 2009, providing a historical cohort.

Results:

Twenty-three questionnaires were evaluated. Response rate of questionnaires for the VU Medical Center was 63% (n=10) and Wilhelmina's Children's Hospital 93% (n=13). No significant differences were found in the mean scores between both groups. However, the untreated group scored more frequently under the -2 SD threshold. In the fine motor skills domain the difference was statistically significant (p=0.031). In the treated group no patients developed cerebral palsy and in the untreated group two patients developed cerebral palsy.

Conclusion:

In this study patients treated with hypothermia tend to have a better neurodevelopmental outcome. No significant differences were found between the two groups, apart from the fine motor skills.

Introduction

Perinatal asphyxia occurs in 1–2/1000 deliveries in the Netherlands. Several parameters may be predicting poor neurodevelopmental outcome, like clinical parameters, neurologic examination, neurophysiology and MRI imaging. Therapeutic hypothermia is a proven therapy to improve outcome in term or near term newborns with perinatal asphyxia.[1] It reduces mortality without increasing disability in this patient group.

In The Netherlands (therapeutic) hypothermia has been introduced in 2008. The introduction of (therapeutic) hypothermia in The Netherlands and Flanders was recently reported showing similar outcome as in previously published studies.[2] However, no comparison has been made to the outcome of patients with perinatal asphyxia without being treated with therapeutic hypothermia.

One method to get insight in child development is the Ages and Stages Questionnaire (ASQ). This is a parent–completed development screener consisting of different age specific questionnaires. It has a high negative predictive value as a screening tool for cognitive and motor developmental delay.[3, 4] The Dutch ASQ for 48 months has been studied for usefulness of detecting early developmental problems and has comparable outcomes in relation to countries worldwide.[5]

The aim of this retrospective study was to examine a historical cohort of patients with perinatal asphyxia of two Neonatal Intensive Care Units (NICU's), of which some have been treated with therapeutic hypothermia to compare with an untreated normothermic control group.

Methods

Since 2008 therapeutic hypothermia as treatment for perinatal asphyxia has been introduced in the NICU's in the Netherlands. It was first introduced in the Wilhelmina Children's Hospital, Utrecht and subsequently in the other hospitals level III NICU's in the Netherlands. A year later, in 2009, it was introduced in VU University Medical Center, Amsterdam. Inclusion criteria for hypothermia treatment, in summary, are gestational age over 36 weeks, signs of perinatal asphyxia (Apgar score <5 at 10 minutes, and/or continued need for resuscitation, and/or pH <7.00 in umbilical cord or within one hour after birth) followed by signs of encephalopathy (e.g. seizures, hypotonia, lethargy).[6] Start of hypothermia had to be within six hours after birth.

Patients admitted to the NICU's of both hospitals in the year 2008 were included in this study, providing a normothermic group of infants from the VU University Medical Center with a hypothermic group of infants from the Wilhelmina Children's Hospital in Utrecht. Parents were contacted to ask for consent to fill out the 48 months ASQ.

The ASQ has five domains: communication, gross motor skills, fine motor skills, problem solving capacity, social emotional development. In each domain six items were scored, as present in the patient, sometimes present or not yet shown by the patient, with respectively 10, 5 and 0 points. If an item was not filled in a score of 0 was imputed, based on the lowest feasible result. A score for each domain, and a total score were calculated. Cut-off thresholds for Dutch children are not yet available, so the American cut-off thresholds have been used. A standard deviation (SD) of less than -1 is considered as a child being at risk of developmental delay, a SD of less than -2 implies developmental delay. The -1 SD thresholds in the five domains are respectively 42, 43, 31, 42 and 38. The -2 SD thresholds are respectively 31, 33, 16, 31 and 27.

Clinical data of all patients were collected, to evaluate if the groups were comparable. Also MRI results were collected.

The study was approved by the Medical Ethics Committee of the VU University Medical Center. Informed consent was obtained.

Statistical analysis was performed using SPSS Software Package version 20. Mean scores on all five domains of the ASQ were compared between the two groups using the Chi square test. A p-value <0.05 was considered statistically significant.

Results

In the Wilhelmina Children's Hospital 14 patients received therapeutic hypothermia, response rate of questionnaires 93% (n=13). In the VU University Medical Center 15 patients would have met the criteria for therapeutic hypothermia (but were not treated), response rate of questionnaires 67% (n=10). In total 23 questionnaires were analysed.

Clinical characteristics, MRI if performed and questionnaire results are stated in *Table 3.1*. The Apgar score at 5 minutes was stated due to absence of 10 minute scores in a quarter of the patients.

No significant differences were found in patient characteristics. Aetiology of perinatal asphyxia was slightly different in both groups. In 15% the aetiology was unknown in both groups. In both groups nuchal cord and shoulder dystocia were frequent causes of the perinatal asphyxia. In the normothermic group meconium aspiration was more common than in the treated group (33% vs 8%).

ASQ

The mean scores of the five domains of the ASQ showed no significant differences between the treated and the normothermic group. However, in the domains of communication and problem solving capacities the hypothermia group tends to have better scores. In none of the domains the mean scores were below the -1 SD threshold. However, when individual patients were analysed half of all infants (11/23) scored in one or more domains below the -1 SD threshold, and one fifth scored below -2 SD (5/23). When comparing the two groups, the patients in the normothermic group scored more often below the -2 SD. Only in the domain of fine motor skills was the difference statistically significant (p=0.031). In the normothermic group two patients developed cerebral palsy. These two patients with cerebral palsy scored lower in all domains of the ASQ. One scored no points in either domain. The other scored in the domains problem solving capacity and social emotional development, but below the -1 and -2 SD threshold respectively, with no additional points in the other domains.

MRI findings

In the normothermic group MRI was performed in seven patients. In three patients the basal ganglia showed abnormalities; two developed cerebral palsy, the other did not. In one patient the posterior limb of the internal capsule was affected without involvement of the basal ganglia. In two patients white matter lesions were found. Of the two patients who developed cerebral palsy, one had a spastic quadriplegia, the other a severe dyskinetic cerebral palsy (both Gross Motor Function Classification System V).

All patients of the hypothermic group had a neonatal MRI. None of the patients showed abnormalities on MRI imaging in the basal ganglia. In five patients white matter lesions were observed. None of the patients developed cerebral palsy. Two patients scored below -2 SD, one for gross motor score, the other on problem solving capacity. The former did not show any abnormalities on MRI imaging, the latter showed abnormalities in the cortex and white matter.

Table 3.1: Results of clinical data and questionnaires

Pt	Gender	GA	BW	UC pH	Lactate	EA	HT	AS 5′	MRI	CP	Com	GM	FM	PO	PS	Total
1	M	42	4380	-	17.0	No	Yes	4	WM	No	55	55	35	60	60	265
2	M	40	3480	-	17.0	Yes	Yes	-	WM	No	60	50	50	60	60	280
3	M	40	3450	-	3.0	Yes	Yes	2	NA	No	40	20	30	50	35	175
4	M	41	5000	-	6.0	Yes	Yes	3	NA	No	55	35	45	60	50	245
5	F	41	3100	-	14.9	No	Yes	3	NA	No	60	60	60	60	60	300
6	M	41	3054	6.70	9.0	No	Yes	1	NA	No	55	40	55	50	45	245
7	M	41	3810	7.10	21.0	No	Yes	5	NA	No	50	40	30	40	60	220
8	F	41	3050	7.00	6.0	No	Yes	4	NA	No	60	60	55	60	60	295
9	M	40	3010	6.90	3.2	Yes	Yes	4	WM/S	No	60	60	70	50	40	250
10	M	40	4000	-	20.0	Yes	Yes	2	WM/C	No	45	45	25	35	25	175
11	M	39	4700	7.10	22.0	No	Yes	3	WM	No	60	50	50	60	50	270
12	M	41	4225	6.80	18.0	Yes	Yes	3	NA	No	55	50	30	50	60	245
13	F	40	3250	-	27.0	-	Yes	-	NA	No	60	60	60	60	60	300
14	M	40	3850	6.98	11.6	Yes	No	4	BGT	Yes	0	0	0	40	20	60

15	F	39	3030	7.25	5.5	Yes	No	1	BGT/WM	Yes	0	0	0	0	0	0
16	M	40	3460	6.97	3.3	Yes	No	6	IC/WM	No	60	60	60	60	60	300
17	M	40	3325	6.85	9.6	No	No	5	NA	No	60	60	60	60	60	300
18	M	38	5295	-	7.6	Yes	No	4	NA	No	45	60	40	40	60	245
19	M	38	3400	6.89	12.0	Yes	No	5	BGT	No	60	55	35	45	60	255
20	F	39	3700	7.47	10.8	Yes	No	4	NA	No	60	60	60	60	60	334
21	M	40	3450	-	16.4	Yes	No	5	NP	No	60	60	60	50	60	290
22	M	38	3965	7.31	2.7	No	No	4	NP	No	50	25	15	45	40	175
23	M	41	3500	-	4.6	No	No	5	NP	No	45	35	35	55	45	215
p-value											0.13	0.41	0.38	0.17	0.51	0.32

Pt, patient; M, male; F, female; GA, gestational age in weeks; BW, birth weight in grams; UC pH, umbilical cord pH; EA, epileptic activity; HT, hypothermia; AS, apgar score; WM, white matter abnormalities; NA, no abnormalities; S, stroke; C, cortex abnormalities; BGT, basal ganglia and thalami abnormalities; IC, internal capsule abnormalities; NP, not performed; Com, communication score; GM, gross motor skills; FM, fine motor skills; PS, problem solving; PE, psycho-emotional
p-value: hypothermia vs normothermia.

3

Discussion

ASQ, in general, is a good screening tool that can be used in a large cohort to screen for developmental delay with little means. Even if MRI showed no or little abnormalities these patients are at risk for neurodevelopmental delay due to perinatal asphyxia. ASQ as a screening tool is easy to use and not time-consuming for parents and doctors. Moreover it can be used on subsequent ages to follow-up the neurodevelopment and it may detect patients with a mild neurodevelopmental delay.

With this limited data set we were able to show that, although the mean scores did not differ between normo- and hypothermic patients, the individual hypothermic patients had better scores on the ASQ. When looking at the patients scoring below -2 SD (implying developmental delay) a significant difference in performance was present to the detriment of the normothermic group.

No previous studies comparing patients with perinatal asphyxia whether or not treated with therapeutic hypothermia have used the ASQ. But these findings are in line with the follow-up studies after therapeutic hypothermia after perinatal asphyxia showing better long term outcome.[1, 7, 8]

Due to the retrospective nature of this study the number of patients could not be expanded. One can assume there might be smaller differences present between the two patient groups, but this could not be detected due to the small study population. In addition a parent-completed questionnaire is a screening tool and less sensitive than a standardized developmental test. Also it is subject to subjectivity of interpretation by parents. This makes it difficult to detect more subtle differences in developmental delay.

As hypothermia was introduced in the VU University Medical Center after 2008 i.e. 2009, in 2008 only patients with respiratory or circulatory insufficiency or neurologic sequelae of perinatal asphyxia e.g. convulsions were admitted to the NICU. One may assume that only more severely affected patients were transferred to the NICU and other less affected patients, however eligible for hypothermia, remained in the referral hospitals. This might explain differences in the aetiology of perinatal asphyxia in the patient population between the two hospitals in 2008. On the other hand, the hypothermic group presented with comparable cardiorespiratory insufficiency and neurologic problems.

In conclusion

When comparing cohort of patients with perinatal asphyxia with the ASQ questionnaire, the patients treated with therapeutic hypothermia tend to have a better outcome at four years of age compared to the normothermic group.

Acknowledgment

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References

1. Azzopardi D, Strohm B, Marlow N, et al. Effects of hypothermia for perinatal asphyxia on childhood outcomes. N Engl J Med. 2014;371(2):140-9.
2. Groenendaal F, Casaer A, Dijkman KP, et al. Introduction of hypothermia for neonates with perinatal asphyxia in the Netherlands and Flanders. Neonatology. 2013;104(1):15-21.
3. Skellern CY, Rogers Y, O'Callaghan MJ. A parent-completed developmental questionnaire: Follow up of ex-premature infants. J Paediatr Child Health. 2001;37:125-9.
4. Squires J, Bricker D, Potter L. Revision of a Parent-Completed Developmental Screening Tool: Ages and Stages Questionnaires. J Pediatr Psychol. 1997;22(3):313-28.
5. Kerstjens JM, Bos AF, ten Vergert EM, et al. Support for the global feasibility of the Ages and Stages Questionnaire as developmental screener. Early Hum Dev. 2009;85(7):443-7.
6. Azzopardi D, Strohm B, Edwards AD, et al. Treatment of asphyxiated newborns with moderate hypothermia in routine clinical practice: how cooling is managed in the UK outside a clinical trial. Arch Dis Child Fetal Neonatal Ed. 2009;94(4):F260-4.
7. Jacobs SE, Berg M, Hunt R, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev. 2013(1):CD003311.
8. Pappas A, Shankaran S, McDonald SA, et al. Cognitive outcomes after neonatal encephalopathy. Pediatrics. 2015;135(3):e624-34.

Part 2

Postnatal predictors

Chapter 4

The prognostic value of NIRS in preterm infants with (suspected) late-onset sepsis in relation to long term outcome: a pilot study

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Abstract

Background and objectives:

Late-onset sepsis is frequently seen in preterm infants and is associated with poor neurodevelopmental outcome. White matter damage is proposed as substrate of poor outcome, with contributing factors as regional hypoxia and effects of cytokines on oligodendrocytes. We investigated the relation between cerebral oxygenation during (suspected) late-onset sepsis and neurodevelopmental outcome.

Methods:

Prospective cohort study, including preterm infants (gestational age <32 weeks and/or birthweight <1500 grams) with (suspected) late-onset sepsis underwent NIRS registration during the first 72 hours of suspected late-onset sepsis. At two years corrected age neurodevelopment was scored using the Bayley Scales of Infant Development-II.

Results:

Thirty-two infants were included. Twenty-seven infants were identified with proven late-onset sepsis and five infants had clinical sepsis without positive blood culture. In this study, late-onset sepsis was predominantly caused by coagulase negative staphylococci (CoNS) (72%). All NIRS values were within normal limits. No association was found between NIRS and impaired neurodevelopmental outcome (n=4) at corrected age two years: composite cognitive score 105 (80–115), composite motor score 103 (82–118) (median and range).

Conclusion:

In this pilot study, late-onset sepsis (predominantly caused by CoNS with a relatively mild clinical course), was not associated with aberrant NIRS values, nor with impaired neurodevelopmental outcome. Further research might establish our findings and elucidate effects of other micro-organisms on cerebral perfusion.

Introduction

Preterm and very low birth weight infants are at risk of acquiring nosocomial sepsis due to their immature immune system.[1] Approximately 21–36% of the admitted infants experience a culture proven late-onset sepsis. Up to 50% of the infants was treated with antibiotics during their Neonatal Intensive Care Unit (NICU) stay, both for blood culture proven and clinically suspected infection.[2, 3]

The long-term effects of sepsis in preterm newborns are well established. Sepsis is associated with adverse long term neurodevelopment, with mental or psychomotor developmental delay.[3] However, also studies have been published, concerning mainly perinatal CoNS infections, in which this relation has not been established.[4]

The adverse long term effects of sepsis are linked to the development of white matter injury,[5, 6] and white matter injury is associated with abnormal psychomotor outcome.[3, 7] One of the mechanisms inducing white matter injury is compromised cerebral blood flow and thus hypoxemia, which can be the result of dysregulation of vital parameters like blood pressure, due to infection. Another mechanism is release of inflammatory mediators, which can induce and increase cerebral damage.[8, 9]

To evaluate effects of nosocomial infection on the brain in preterm infants, cerebral imaging by cerebral ultrasounds or MRI can be performed. However, cerebral ultrasound will only show already established gross brain damage. Other modalities of brain monitoring might provide an additional value, e.g. by detecting changes in cerebral perfusion, which may prelude cerebral damage.

Several studies have determined reference values of regional cerebral oxygen saturation in preterm infants.[10-12] Also studies concerning the association between regional cerebral oxygen saturation and long term outcome become more and more available. However, most studies report the regional cerebral oxygenation during the first 72 hours of life. Alderliesten et al. showed an association between lower regional cerebral oxygen saturation in the first 72 hours of life and lower neurodevelopmental outcome scores.[13] In another follow-up study, this association could not be demonstrated.[14]

Van der Laan et al. studied multisite fractional tissue oxygen extraction during an episode of clinical sepsis on short term outcome.[15] The effects on cerebral oxygenation during late-onset sepsis, and in relation to long term neurodevelopmental outcome, have not been described previously. In search for a causal link, it would be interesting to investigate whether impairments in cerebral autoregulation might be of influence in development of white matter injury and relate to subsequent long term development. One could hypothesize cerebral oxygenation is compromised during late-onset sepsis due to instability of the infant during the first days after onset and thereby influence long term outcome.

Aim of this study was to investigate the effects of late-onset sepsis on cerebral oxygenation during the first 72 hours after onset and the relation with psychomotor outcome at corrected age of two years.

Methods

We performed a prospective, observational cohort study: the INFANT study. Data of preterm infants <32 weeks gestational age and/or <1500 grams admitted to the level III Neonatal Intensive Care Unit (NICU) of the VU Medical Center between August 2012 and December 2014 and suspected of late-onset sepsis were prospectively collected. Preterm infants with syndromal or chromosomal abnormalities and congenital metabolic disorders were excluded. Informed parental consent was obtained and approval was given by the medical ethical committee of the VU University Medical Center (protocol number 2008/77).

Late-onset sepsis was suspected when one of the following clinical symptoms occurred: hypothermia (<36.5 °C) or hyperthermia (>37.5 °C), hypotension, tachycardia, apnea, feeding problems, irritability and/or apathy. Late-onset sepsis was defined as a positive blood culture after 72 hours of life.[16, 17] When blood culture remained sterile, but antibiotic treatment was given for seven days due to persistent clinical symptoms, late-onset sepsis was considered probable and defined as clinical sepsis.

Clinical data were collected from patients' medical charts. Neonatal variables included gestational age at birth, birth weight, gender, intraventricular hemorrhage according to Volpe18 and white matter echogenicity on cerebral ultrasound defined as any white matter hyperechogenicity occurring during the study period on any localization in the cerebrum, need for inotropic support, need for mechanical ventilation and lumbar puncture.

During the first 72 hours of suspicion of late-onset sepsis, cerebral oxygenation was registered by INVOS 5100C near infrared spectrometer (Covidien/ Medtronic, Boulder, Colorado, USA) in combination with the small adult sensor Somasensors with emitter-detectors distance of 3 and 4 cm (Covidien/ Medtronic) with data storage every 34 seconds. The sensor was placed left or right frontoparietal on the patients head providing regional cerebral oxygenation ($rScO_2$) and replaced every three hours to prevent skin lesions. Change of position of the sensor might alter the $rScO_2$ value measured, however Hyttel-Sorensen et al. state out of range values on average will tend to normalize.[19] Isolated drop out data after repositioning of the sensor were removed. Arterial oxygen saturation (SaO_2) was retrieved by pulse oximetry on a limb. To investigate the balance between oxygen delivery and oxygen consumption, the relative cerebral fractional tissue oxygen extraction (cFTOE) can be formulated as a ratio: $(SaO_2\text{-}rScO_2)/ SaO_2$. Analyses were performed in time frames of eight-hour duration. Artifacts in registrations were assessed. If a value of zero was registered, this value was eliminated from the data set except for severely respiratory or circulatory compromises patients, which could correspond with a value of zero. All other values were maintained.

Cognitive and motor development was scored at two years corrected age by the Bayley Scales of Infant Development II (BSID-II), consisting of a mental developmental index, psychomotor developmental index and a behavioral rating scale.[20] The outcome was rated good when BSID-II >85 and poor if BSID-II<85 or non-survived. Also language skills were assessed by the lexi quotient.[21, 22]

Statistical analysis was performed with SPSS using version 22 (SPSS Inc, Chicago, Illinois, USA). For multivariate analysis we used the non-parametric Kruskal Wallis test and if appropriate the Mann-Whitney U test. Due to the non-normality of the data these non-parametric tests were chosen. Mixed models were used as appropriate for longitudinal data analysis. A probability p-value <0.05 was considered statistically significant.

Results

During the study period, 32 infants (14 boys, 18 girls) were included. Patient characteristics are shown in *Table 4.1* and presenting clinical symptoms are stated in *Table 4.2*. Twenty-

Table 4.1: Patient characteristics

Clinical characteristics	N (%) or median (range)
Gender: boys	14 (44%)
Gestational age, weeks	27 4/7 (24 0/7 – 32 0/7)
Birth weight, grams	1029 (545 – 1900)
Survival	31 (97%)
Postnatal day of onset late-onset sepsis	10 (4 – 48)
Lumbar puncture	23 (72%)
Need for intubation during LOS	8 (25%)
Need for inotropic support	2 (6%)
Signs of NEC stage II/III during episode of LOS	2 (6%)
White matter abnormalities on cerebral ultrasound	4 (13%)

LOS, late-onset sepsis, gestational age and birthweight stated as median and range.

Table 4.2: Presenting clinical symptoms

Symptoms	N (%)
Apnea/ increased need of oxygen	24 (75%)
Apathy	19 (59%)
Tachycardia	13 (41%)
Temperature-instability	11 (34%)
Feeding problems	10 (31%)
Hyperglycemia	2 (6%)
Hypotension	1 (3%)

seven infants were identified with proven late-onset sepsis and five infants had clinical symptoms, elevated CRP and were treated with antibiotics for seven days, but without positive blood culture. Three infants experienced two episodes of late-onset sepsis suspicion. Only the first episode was used for analysis. CoNS (n=23) were the causal agents in 72% in cases with proven late-onset sepsis. *Staphylococcus aureus* (n=3) and *Escherichia coli* (n=1) counted for the other proven late-onset sepsis episodes.

Figure 4.1 shows the mean values of $rScO_2$ and cFTOE in the first 72 hours of late-onset sepsis. The median postnatal day of onset late-onset sepsis was day 10 (range day 4 to 48). Longitudinal analysis shows no significant difference over the 72-hour registration, either for $rScO_2$ nor FTOE (respectively p=0.536 and p=0.588).

Cognitive and motor development was assessed at corrected age of two years. One patient of 32 patients died during NICU admittance. Twenty-eight of 31 patients who survived

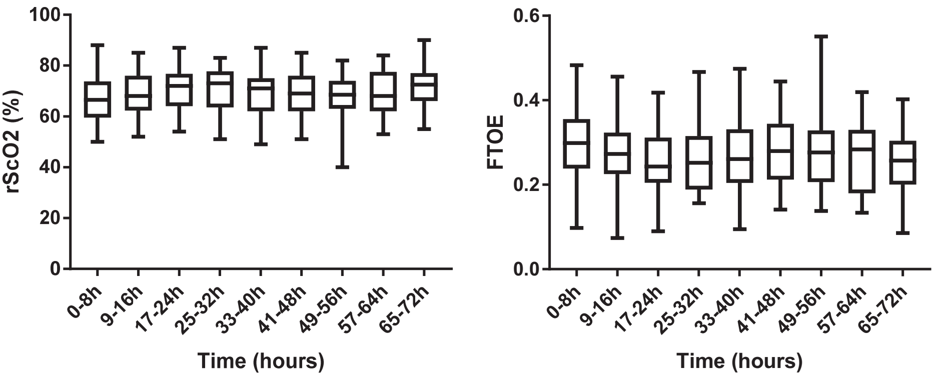

Figure 4.1: Mean $rScO_2$ and FTOE during first three days of late-onset sepsis.
$rScO_2$, regional cerebral oxygenation saturation; cFTOE, cerebral fractional tissue oxygen extraction; h, hours.

were tested (90%). Three patients were lost to follow-up due to them moving abroad (1), cancellation of the test due to personal circumstances (1) and one patient turned out to have a severe structural cerebral disorder (double cortex) for which this patient was excluded for follow up analysis. Results of the median cognitive and motor development were within normal range (*Table 4.3*). Though one patient scored under 85 for cognitive development (score 80) and two patients below 85 for motor development (score for both patients 82). Also lexi quotient and total behavioral scores are within normal range.

There was no correlation between lowest mean value of $rScO_2$ during the 72 hour period and the composite cognitive score (*Figure 4.2*).

Brain ultrasound abnormalities occurred in four patients. $rScO_2$ and FTOE in the first 72 hours of late-onset sepsis were not associated with echo densities on cerebral ultrasound in any localization in the cerebrum, nor with good outcome (BSID-II score mental and motor ≥-1 SD) nor with poor outcome (BSID-II either mental or motor <-1SD, or non-survived).

Table 4.3: Median corrected age cognitive score, motor score, total behavioral score and lexi quotient

Score	Median (range)
Corrected age at testing (range)	24m 4d (23m 3d – 31m 0d)
Corrected age composite cognitive score (range)	105 (80 – 115)
Corrected age composite motor score (range)	103 (82 – 118)
Total behavioral score (range)	21 (3 – 56)
Lexi quotient (range)	92 (61 – 119)

m, months; d, days.

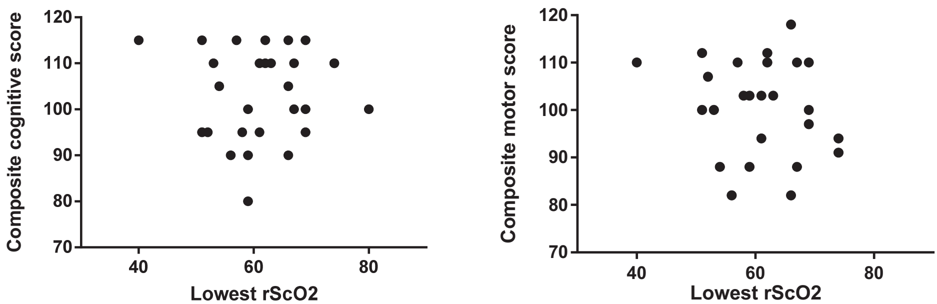

Figure 4.2: Lowest $rScO_2$ (8 hour interval) and composite cognitive and motor outcome based on the Bayley's Scales of Infant Development II.
$rScO_2$, regional cerebral oxygenation saturation.

Discussion

During the first 72 hours of late-onset sepsis we did not detected changes in cerebral tissue oxygenation and consumption as measured by NIRS in our study cohort of preterm infants in comparison to the reference values the first days after birth as presented by Alderliesten et al.[10] as best available reference values at this moment. Other studies report data regarding cerebral oxygenation in subsequent days after birth showing a small decline in $rScO_2$ values, however these studies had small study populations.[11] Due to possible small alterations in cerebral perfusion during late-onset sepsis which could not be demonstrated in this study population, a larger cohort is required to elucidate the effect of late-onset sepsis on cerebral perfusion. Also, to assess the association of regional cerebral oxygen saturation and long term neurodevelopmental outcome larger cohorts are needed.[19]

In this cohort CoNS were highly represented, 72% of all proven infections, which is in line with previously published results.[23] CoNS infections are known to have a relatively mild clinical presentation and are not associated with poor motor outcome.[4] In line with the relatively good prognosis of CoNS infections we did not find brain damage in terms of white matter abnormalities in this cohort using cerebral ultrasound.[24] We could not establish an effect on cerebral oxygenation. The latter might be explained by cerebral autoregulation not being compromised during CoNS sepsis. In addition, with NIRS monitoring cerebral oxygenation is measured more superficially including the cortex, but excluding a major part of the white matter. Only two infants needed inotropic support suggesting that the cerebral tissue oxygenation is compliant enough to warrant appropriate cerebral oxygenation in these infants.

The association of white matter injury and decreased values of $rScO_2$ has been described in infants with congenital heart disease.[25] It is also important to consider that an increase of cFTOE ratio might either indicate a reduced oxygen delivery to the brain with a constant oxygen consumption of the brain, or a higher oxygen consumption than delivery[26] and a possible indicator for ominous white matter injury. In this study cerebral ultrasound has been used as a modality of imaging, which is available in the acute period of infection, and might index patients at risk for white matter injury. When exploring sequelae of cerebral hypoperfusion in terms of white matter abnormalities on ultrasound no significant differences were found in $rScO_2$ or cFTOE.

In contrast to most findings, Alshaikh et al. reported that CoNS infections were associated with a cognitive delay at 36 months corrected age.[27] However, we found in this cohort the BSID-II at 24 months corrected age median composite scores for cognitive and motor development were in line with the general population. Three individual patients scored less than 85 for either cognitive or motor development at the BSID-II test. Also when exploring the lowest $rScO_2$ values during the infectious episode no relation could be demonstrated with cognitive or motor outcome. Taken into account it is a relatively small study cohort smaller differences between the CoNS group and the general population might have remained undetected, and therefore the relation between CoNS, $rScO_2$, and adversed long term outcome cannot be rejected. Also, follow-up at school age might provide more insight in more subtle cognitive disturbances due to late-onset sepsis. On the other hand, it might also be the representation of a relatively mild course of a CoNS sepsis. Further studies should be performed to determine if the hypothesis "disturbed cerebral oxygenation might be related to adverse long term outcome in CoNS sepsis" can be proven or should be rejected.

In our cohort we also explored the relation between $rScO_2$ and cFTOE and a good (BSID-II score mental and motor ≥-1 SD) or a poor outcome (BSID-II either mental or motor <-1 SD, or non-survived). We were not able to show any relation between NIRS values in the first 72 hours during late onset infection and neurodevelopmental outcome at two years. However, as CoNS infections are the most frequent etiology of late-onset sepsis, it would be interesting to explore the effects of micro-organisms other than CoNS in a larger cohort than this study provides.

We do acknowledge limitations of our study. This is a single center study in a relatively small group of preterm infants. It would be interesting to study a larger cohort and compare the cerebral oxygenation values and long term follow up to a control group and evaluate other factors influencing cerebral oxygenation and the effects on long term outcome.

Furthermore, the high incidence of coagulase-negative staphylococci limited the number of patients with late-onset sepsis caused by other bacteria. Therefore, it is difficult to assess if cerebral tissue oxygenation is influenced differently during late-onset sepsis caused by different micro-organisms like gram-negative bacteria. And if so, if there is any difference in disturbance of cerebral tissue oxygenation in the relatively mild course of CoNS versus gram negative bacteria.

NIRS monitoring registers the mixed oxygen saturation from superficial tissue e.g. the cerebral cortex and part of the white matter. Also, it is not certain whether white matter and deep grey matter were equally well represented in the registered values of cerebral oxygenation.[28] Therefore, conclusion concerning the relation between white matter abnormalities and NIRS measured oxygen consumption should be drawn with care.

In conclusion

In this pilot study we did not detect any changes in cerebral oxygenation during the first 72 hours of late-onset sepsis. In this small cohort we did not detect an effect of late-onset sepsis on neurodevelopmental outcome at two years corrected age. However, this might be the result of a relatively mild course of late-onset sepsis by CoNS. Further research could elucidate the effect of other causal micro-organisms.

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References

1. Melville JM, Moss TJ. The immune consequences of preterm birth. Front Neurosci. 2013;7:79.
2. Adams-Chapman I, Stoll BJ. Neonatal infection and long-term neurodevelopmental outcome in the preterm infant. Curr Opin Infect Dis. 2006;19(3):290-7.
3. Adams-Chapman I. Long-term impact of infection on the preterm neonate. Semin Perinatol. 2012;36(6):462-70.
4. Mittendorf R, Covert R, Kohn J, et al. The Association of Coagulase-Negative Staphylococci Isolated from the Chorioamnion at Delivery and Subsequent Development of Cerebral Palsy. J Perinatol. 2001;1:3-8.
5. Perlman J. White matter injury in the preterm infant: an important determination of abnormal neurodevelopmental outcome. Early Human Development. 1998;53(2):99-120.
6. Saliba E, Marret S. Cerebral white matter damage in the preterm infant: pathophysiology and risk factors. Semin Neonatol. 2001;6(2):121-33.
7. Mitha A, Foix-L'Helias L, Arnaud C, et al. Neonatal infection and 5-year neurodevelopmental outcome of very preterm infants. Pediatrics. 2013;132(2):e372-e80.
8. Chau V, Brant R, Poskitt KJ, et al. Postnatal infection is associated with widespread abnormalities of brain development in premature newborns. Pediatr Res. 2012;71(3):274-9.
9. Glass HC, Bonifacio SL, Chau V, et al. Recurrent postnatal infections are associated with progressive white matter injury in premature infants. Pediatrics. 2008;122(2):299-305.
10. Alderliesten T, Dix L, Baerts W, et al. Reference values of regional cerebral oxygen saturation during the first 3 days of life in preterm neonates. Pediatr Res. 2016;79(1-1):55-64.
11. McNeill S, Gatenby JC, McElroy S, et al. Normal cerebral, renal and abdominal regional oxygen saturations using near-infrared spectroscopy in preterm infants. J Perinatol. 2011;31(1):51-7.
12. Pichler G, Binder C, Avian A, et al. Reference ranges for regional cerebral tissue oxygen saturation and fractional oxygen extraction in neonates during immediate transition after birth. J Pediatr. 2013;163(6):1558-63.
13. Alderliesten T, Lemmers PM, van Haastert IC, et al. Hypotension in preterm neonates: low blood pressure alone does not affect neurodevelopmental outcome. J Pediatr. 2014;164(5):986-91.
14. Plomgaard AM, Alderliesten T, van Bel F, et al. No neurodevelopmental benefit of cerebral oximetry in the first randomised trial (SafeBoosC II) in preterm infants during the first days of life. Acta Paediatr. 2019;108(2):275-81.

15. van der Laan ME, Schat TE, Olthuis AJ, et al. The Association between Multisite Near-Infrared Spectroscopy and Routine Hemodynamic Measurements in Relation to Short-Term Outcome in Preterms with Clinical Sepsis. Neonatology. 2015;108(4):297-304.
16. van der Zwet WC, Kaiser AM, van Elburg RM, et al. Nosocomial infections in a Dutch neonatal intensive care unit: surveillance study with definitions for infection specifically adapted for neonates. J Hosp Infect. 2005;61(4):300-11.
17. Haque KN. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med. 2005; 6(3 Suppl):S45-9.
18. Volpe JJ. Neurology of the Newborn. Philedelphia: Saunders Elsevier; 2008.
19. Hyttel-Sorensen S, Pellicer A, Alderliesten T, et al. Cerebral near infrared spectroscopy oximetry in extremely preterm infants: phase II randomised clinical trial. BMJ. 2015;350(jan05 2):g7635-g.
20. Bode MM, D'Eugenio DB, Mettelman BB, et al. Predictive Validity of the Bayley, Third Edition at 2 Years for Intelligence Quotient at 4 Years in Preterm Infants. J Dev Behav Pediatr. 2014; 35:570-5.
21. Schlichting JEPT, Spelberg HC . Lexilijst Nederlands. Een instrument om de taalontwikkeling te onderzoeken bij Nederlandstalige kinderen van 15-27 maanden in het kader van vroegtijdige onderkenning. Amsterdam: Pearson Assessment & Information B.V.; 2002.
22. Schlichting JEPT. Lexiconlijsten Marokkaans-Arabisch, Tarifit-Berbers en Turks. Instrumenten om de taalontwikkeling te onderzoeken bij jonge Marokaanse en Turkse kinderen in Nederland. Handleiding. Amsterdam: JIP-Uitgeverij; 2006.
23. Boghossian NS, Page GP, Bell EF, et al. Late-onset sepsis in very low birth weight infants from singleton and multiple-gestation births. J Pediatr. 2013;162(6):1120-4, 4 e1.
24. Claessens LC, Zonnenberg IA, van den Dungen FA, et al. Cerebral ultrasound abnormalities in preterm infants caused by late-onset sepsis. PLoS One. 2017;12(3):e0173227.
25. Mebius MJ, Kooi EMW, Bilardo CM, et al. Brain Injury and Neurodevelopmental Outcome in Congenitla Heart Disease: A Systematic Review. Pediatrics. 2017;140(1):e20164055.
26. Lemmers PM, Molenschot MC, Evens J, et al. Is cerebral oxygen supply compromised in preterm infants undergoing surgical closure for patent ductus arteriosus? Arch Dis Child Fetal Neonatal Ed. 2010;95(6):F429-34.
27. Alshaikh B, Yee W, Lodha A, et al. Coagulase-negative staphylococcus sepsis in preterm infants and long-term neurodevelopmental outcome. J Perinatol. 2014;34(2):125-9.
28. Greissen G, Børch K. White Matter Injury in the Preterm Neonate: The Role of Perfusion. Dev Neurosci. 2001;23:209-12.

Chapter 5

Cerebral ultrasound abnormalities in preterm infants caused by late-onset sepsis

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Abstract

Introduction:

This study describes cerebral ultrasound abnormalities caused by late-onset sepsis (LOS) in very preterm infants with a gestational age of <32 weeks and/or birthweight <1500 grams.

Methods:

The prospective study ("INFANT study") included 117 preterm infants with suspected LOS. Proven LOS was defined as a positive blood culture after 72 hours of life. In case of coagulase-negative staphylococci an elevated C-reactive protein was additionally required to establish proven LOS. Patients were identified as proven LOS and patients with only clinical symptoms of LOS. Cerebral ultrasound images were obtained in the first week after birth, during/after LOS and before discharge. Cerebral findings were divided in no/minor and major abnormalities.

Results:

Eighty-six preterm infants had proven LOS and 31 preterm infants had only clinical signs of LOS. Four infants were excluded because pre-existing major brain abnormalities. No significant differences (p=0.624) for incidence of major brain abnormalities on cerebral ultrasound were found.

Conclusion:

No differences were revealed in prevalence of major brain abnormalities between the groups with proven LOS and with clinical signs of LOS. Both infants with a gram negative sepsis developed major brain abnormalities, whereas only three of 66 preterm infants coagulase-negative staphylococci sepsis developed major brain abnormalities.

Introduction

Late-onset sepsis (LOS) has a large impact on the neurodevelopment of very preterm infants (gestational age <32 weeks) and/or very low birth weight infants (birth weight <1500 gram) characterized by an increased risk for developing cognitive impairments, cerebral palsy and other neurodevelopmental disabilities compared to preterm infants without a sepsis.[1-5] Earlier studies have shown that up to 40% of the very preterm infants (born <32 weeks gestational age) had at least one episode of LOS during their Neonatal Intensive Care Unit (NICU) stay.[6-9] Moreover, infants with a birth weight <1000 grams were more likely to develop an infection.[9] Overall, half of the preterm infants with clinical symptoms of LOS showed a positive blood culture, in most cases coagulase-negative staphylococci (CoNS).[2, 7, 10]

Several studies have revealed that preterm infants with a proven infection were more likely to develop periventricular leukomalacia (PVL).[1, 3] PVL grade II and III (cystic form), associated with the worst neurodevelopmental outcome, occurs in up to 1.3 % of the preterm infants and is a strong predictor of cerebral palsy.[1, 11] A more common brain injury is the germinal/intra ventricular hemorrhage (IVH) that affects 30% of the preterm infants.[12] IVH can be complicated by post-hemorrhagic ventricular dilation or periventricular venous infarction. These complications are related to cerebral palsy and cognitive impairment.[13-14]

Transient abnormalities and changes of the preterm brain during an infection episode have not been fully described yet. Moreover, it is important to know how different bacterial agents causing LOS may act on the developing preterm brain. Short-term abnormalities can be diagnosed using ultrasonography or MRI. MRI as a neuroimaging technique is preferred because of its high sensitivity and specificity, but has also disadvantages for a preterm infant. MRI cannot be performed bedside and the use of sedatives is frequently necessary. Ultrasound as a neuroimaging technique, however, has the advantage it can easily be performed longitudinally and bedside.

The aim of this study was to investigate the abnormalities seen on cerebral ultrasound during and after LOS.

Methods

Study population

Preterm infants with a gestational age of <32 weeks and/or birth weight <1500 gram admitted to the level III NICU of the VU University Medical Center between March

2008 and December 2014. Parents were asked for informed consent to participate in the "INFANT" study, a prospective study investigating immunogenetic, pharmacological and neurodevelopmental aspects of LOS and meningitis in preterm infants. All preterm infants with a suspected LOS were prospectively included. Infants with syndromal or chromosomal abnormalities and congenital metabolic disorders were excluded. Informed parental written consent was obtained and approval was given by the medical ethical committee of the VU University Medical Center. An observational study was performed, due to a lack of information on the outcome parameter in this age group it was not possible to perform a power calculation to calculate sample size.

Late-onset sepsis

LOS was suspected when one of the following clinical symptoms occurred: hypothermia (<36.5 °C) or hyperthermia (>37.5 °C) or temperature instability, hypotension, tachycardia, apnea, feeding problems, irritability and/or apathy. Proven LOS was defined as a positive blood culture (BACTEC Peds Plus™/F Medium; Becton Dickinson) after 72 hours of life. During analysis, if PCR was available for research purposes, it was used to differentiate between causal agents. In case of CoNS an elevated C-reactive protein within two days of blood culture was additionally required for a final diagnosis. LOS was considered proven if blood culture and/ or PCR turned positive. When blood culture did not turn positive but the clinical signs implied antibiotic treatment for seven days, LOS was considered probable but not proven.[4, 9]

All preterm infants who were suspected for LOS received empirical treatment with intravenously administered amikacin and penicillin G for at least two days. Depending on the causal agent found by blood culture, this combination of antibiotics was continued or was switched to another antibiotic regime. Antibiotics were discontinued, when blood culture was sterile and CRP was not elevated. However, in case of clinical LOS or elevated CRP, empirical antibiotics were continued for seven days.

Cerebral ultrasound

Cerebral ultrasound images, which were performed as standard of care, were retrospectively collected from the medical chart of the preterm infant and the database of the Radiology Department and analyzed. Cerebral ultrasound was performed using a real-time scanner (AlokaProsound α7, Aloka co., Ltd., Tokyo, Japan). Images were obtained using a 7.5 MHz transducer. Images were recorded in at least five coronal and five sagittal planes using the anterior fontanel as an acoustic window. In addition, the mastoid fontanel was used as an acoustic window for observing the midbrain, posterior fossa and the ventricular system. Preterm infants with major brain abnormalities seen on ultrasound in the first week after birth were excluded from analysis.

During their stay in the NICU, all preterm infants underwent at least three cerebral ultrasounds. One cerebral ultrasound was performed in the first week after birth by a pediatric radiologist. The attending neonatologist performed sequential cerebral ultrasounds. At least one cerebral ultrasound was performed during LOS. The last cerebral ultrasound was performed before discharge. To identify ultrasound abnormalities these findings had to be seen in at least two planes. These abnormalities were divided in no or mild abnormalities and major abnormalities.

Grading of the intraventricular hemorrhage (IVH) was according to Volpe[15] and periventricular leukomalacia (PVL) according to de Vries et al.[16] Mild abnormalities included IVH grade I (germinal matrix hemorrhage), grade II (10–50% of the lateral ventricle is filled with blood) and PVL grade I (transient echogenicity at least for seven days), inhomogeneous and/or seriously increased echogenicity, plexus cysts. *Figure 5.1A* shows an example of mild PVL/echodensities. Major abnormalities included abnormalities of the basal ganglia, IVH grade III (>50% of the lateral ventricle is filled with blood, usually with distension of the ventricle), periventricular venous infarction, post-hemorrhagic ventricular dilation, PVL grade II (small fronto-parietal cystic lesions), grade III (extensive periventricular cystic lesions) and grade IV (extensive cystic lesions in the deep white matter). *Figure 5.1B* shows an example of cystic PVL. Images were scored in consensus by IZ and LC.

5

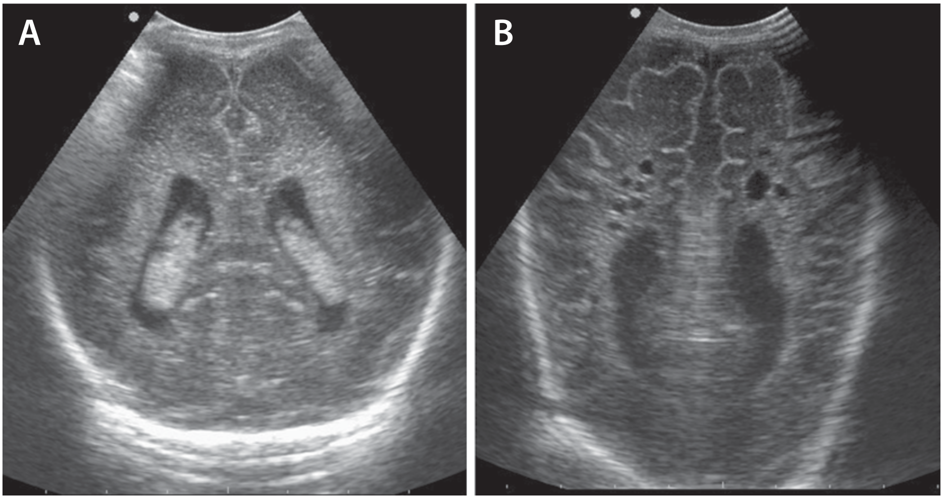

Figure 5.1: Minor and major brain abnormality on ultrasound images: A) Periventricular echodensities/ mild PVL (grade 1); B) Cystic form of PVL.

Clinical data

Clinical data were collected from the patients' medical charts. Maternal variables included maternal pre-eclampsia/HELLP syndrome, chorioamnionitis, maternal group B streptococcal infection, usage of steroids and premature prolonged rupture of membranes. Neonatal variables included gestational age at birth, birth weight, gender, CRIB-score I (clinical risk index for babies), days of ventilation, >24 hours steroid treatment for either respiratory or circulatory insufficiency, lumbar puncture for diagnostic reasons, hemodynamic significant patent ductus arteriosus (HS-PDA), surgery, necrotizing enterocolitis (NEC) stage II or III according to Bell,[17] duration of admittance and death.

Statistical analysis

Data were analyzed with SPSS using version 21 (SPSS Inc, Chicago, Illinois, USA). Statistical analyses were performed with Chi-square using Fisher's exact test as appropriate for dichotomous data. A probability p-value <0.05 was considered statistically significant.

Results

Patient characteristics

During the study-period, 117 preterm infants (58 male, 59 female) were included. Eighty-six preterm infants were identified with a proven LOS and 31 preterm infants only had clinical symptoms of LOS, as described before. The most frequently observed symptoms were apnea, apathy and temperature instability. CoNS (n=67) were the causal agents in 78% of LOS. *Staphylococcus aureus* (n=12), *Escherichia coli* (n=2), *Streptococcus agalactiae* (n=1), *Klebsiella oxytoca* (n=1), Gram positive rod (not further specified) (n=1) and a mixture of CoNS and *Staphylococcus aureus* (n=2) were the remaining causal agents. In total six preterm infants had a positive cerebrospinal fluid culture. In four infants *Staphylococcus aureus* (n=3) or *Escherichia coli* (n=1) was found, suggesting meningitis. In two CoNS were found, possibly due to contamination.

The characteristics of the maternal and neonatal data of both groups are shown in *Table 5.1*, showing only significant differences between type of infection groups in gender, confirming the overrepresentation of CoNS infection in the study group.

Table 5.1: Patient characteristics

Clinical parameters	Proven LOS (n=86)			
	CNS (n=67)	Others (n=19)	Clinical LOS (n=31)	p- value
Maternal characteristics				
Maternal chorioamnionitis, n (%)	18 (27)	6 (32)	10 (32)	0.815
Pre-eclampsia/HELLP, n (%)	20(30)	7(37)	10(32)	0.855
GBS infection, n (%)	5 (7)	1 (5)	2 (6)	0.938
Antenatal steroids, n (%)	58 (87)	18 (95)	27 (87)	0.687
Premature prolonged rupture of membranes, n (%)	8 (12)	4 (21)	5 (16)	0.589
Neonatal characteristics				
Male gender, n (%)	37 (55)	6 (32)	15 (48)	<0.001
Female gender, n (%)	30 (45)	13 (68)	16 (52)	<0.001
Gestational age in weeks, mean (range)	28 (24–32)	27 (24–33)	27 (24–31)	0.769
Birth weight, mean (range)	1112 (545–1965)	960 (550–1800)	1011 (405–1855)	0.375
Lumbar puncture, n (%)	41 (61)	13 (68)	17 (55)	0.629
CRIB, mean(range)	3 (0–15)	4 (0-12)	5 (0–12)	0.484
Respiratory support (days), mean (range)	11 (0–87)	13 (0–44)	12 (0–51)	0.255
Steroid therapy >24 hours, n (%)	10 (15)	3 (16)	4 (13)	0.979
NEC grade II/III, n (%)	11 (16)	5 (26)	4 (13)	0.462
Hemodynamic significant PDA, n (%)	17 (25)	5 (26)	11 (35)	0.574
Surgery, n (%)	4 (6)	3 (16)	2 (6)	0.350
Duration of admission (days), mean (range)	39 (10–116)	41 (6–105)	41 (8–91)	0.073
Died during admission, n (%)	5 (7)	4 (21)	3 (12)	0.350

LOS, late-onset sepsis; HELLP, Hemolysis Elevated Liver Enzymes and Low Platelets; GBS, group B Streptococcal; NEC, Necrotizing enterocolitis; PDA, patent ductus arteriosus; n, number.

5

Major abnormalities on cerebral ultrasound

In total, 409 cerebral ultrasounds (mean 3 per infant, range 2–5) were collected. Mild white matter abnormalities were seen in approximately one third of the patients, both in the proven and clinical LOS group.

The initial cerebral ultrasound (n=114) performed in the first week of life showed major abnormalities in four out of 114 preterm infants. These four infants showed an intraventricular hemorrhage with venous infarction and were excluded for analysis. In none of these infants brain abnormalities worsened after an infection episode. Two of these four infants died due to redirection of care due to the combination of severe cerebral abnormalities and multiple organ failure following the infectious episode. In three infants no initial ultrasound was available. An overview of all brain abnormalities is shown in *Table 5.2*.

Table 5.2: Brain abnormalities seen on cerebral ultrasound during and/or after LOS*

	Proven LOS (n=84)		Clinical LOS (n=29)
	CNS (n=66)	Others (n=18)	
IVH grade I (n)	6	4	2
IVH grade II (n)	5	1	1
IVH grade III (n)	1	1	1
IVH grade IV (n)	0	0	0
PHVD (n)	0	1	0
PVL grade I (n)	23	4	10
PVL grade II (n)	0	0	0
PVL grade III (n)	0	0	0
PVL grade IV (n)	0	0	1
Echodensities of thalamus/ basal ganglia(n)	2	2	0

* Preterm infants with major brain abnormalities in the first week after birth were excluded.
LOS, late-onset sepsis; CNS, coagulase-negative staphylococci; IVH, intra ventricular hemorrhage; PHVD, posthemorrhagic ventricular dilation; PVL, periventricular leukomalacia; n, number.

Nine preterm infants, two with only clinical symptoms of LOS and seven with a proven LOS (six with positive blood culture and/ or PCR, one with positive PCR), developed major brain abnormalities during or following their infectious episode (*Tables 5.3 and 5.4*). Two of these infants developed major brain abnormalities two days prior to the suspected infectious episode.

Table 5.3: Major brain abnormalities seen on cerebral ultrasound during and/or after LOS*

Case number	Infection	Major abnormalities*	PCR confirmed
# 17	*Staphylococcus aureus*	Infarction of the thalamus Intraventricular hemorrhage Grade II	Yes
# 22	*Escherichia coli*	Intraventricular hemorrhage Grade III Posthemorrhagic ventricular dilatation	Yes
# 33	Coagulase-negative staphylococci	Echodensities thalamus	Yes
# 49	*Streptococcus agalactiae*	Ventriculitis Widening of the ventricles	Yes
# 50	*Staphylococcus aureus*	Intraventricular hemorrhage Grade I Infarction of the thalamus	Yes
# 72	Coagulase negative staphylococci	Echodensities of the basal ganglia Periventricular leukomalacia Grade I Loss of white matter Widening of the ventricles	Yes
# 97	Coagulase negative staphylococci	Intraventricular hemorrhage Grade III	No PCR available

* Preterm infants with major brain abnormalities in the first week after birth were excluded.

Table 5.4: Major brain abnormalities seen on cerebral ultrasound during and/or after an episode of clinical symptoms of LOS, with a negative blood culture and negative PCR

Case number	Major abnormalities*
# 26	Intra ventricular hemorrhage Grade III + IV Venous infarction Widening of the ventricles
# 35	Periventricular leukomalacia Grade IV Loss of white matter

In the group of infants who developed major brain abnormalities during or following LOS (n=7) blood culture revealed *Staphylococcus aureus* (n=2), CoNS (n=3) and *Escherichia coli* (n=1). One preterm infant had both LOS caused by *Streptococcus agalactiae* and meningitis with *Escherichia coli* as causal agent.

Both patients with LOS caused by *Staphylococcus aureus* (n=2) showed echodensities of the thalamus, which was an indication of a thalamus infarction. This was confirmed with MRI, which was performed at 40 weeks gestational age of the preterm infant. In both patients had iv catheters, but no thrombi on the tip or nearby the tip were found when investigated by ultrasound.

In addition, the infants with a proven CoNS LOS (n=3) showed in one infant an intraventricular hemorrhage grade III and in two infants echodensities of the basal ganglia. In all these infants the abnormalities were observed after onset of the infectious episode. One of them developed a periventricular leukomalacia and loss of white matter tissue and eventually died due to therapy resistant respiratory insufficiency. In the other infant, the echodensities of the basal ganglia, seen during the infectious episode on ultrasound, were no longer visible on MRI at term age. Escherichia coli was isolated from cerebrospinal fluid indicating meningitis in combination with LOS caused by *Streptococcus agalactiae* in one patient. Another infant with proven LOS with *Escherichia coli* meningitis was suspected, however cerebrospinal fluid remained sterile. On ultrasound both infants presented the image of ventriculitis and ventricular hemorrhage resulting in dilatation of the ventricles.

In the group with only clinical symptoms of LOS (n=31), two preterm infants developed major brain abnormalities on cerebral ultrasound; one infant developed intraventricular hemorrhage bilaterally with venous infarction and ventricle dilatation, that occurred two days prior to the presentation of clinical symptoms of infection; one infant developed cystic PVL four weeks after the infectious episode (*Table 5.3*).

Overall, during and/or after an infection episode no significant differences (p=0.624) were found in incidence of major abnormalities detected on cerebral ultrasound between the group of preterm infants with a proven LOS and the group with a probable LOS (clinical symptoms but negative blood culture).

Discussion

This study shows that during a sepsis episode there is no difference in major brain ultrasound abnormalities between infants with a proven LOS and infants with only clinical symptoms of LOS. In two infants abnormalities were found prior to the sepsis episode, which might suggest other mechanisms might be of importance. Mild white matter abnormalities are present in one third of all patients.

The overall findings of the current study are not in line with earlier studies, indicating that culture positive sepsis is an important risk factor for ventricular dilation, periventricular leukomalacia (PVL) and intraventricular hemorrhage (IVH) in preterm infants.[3, 4, 18-20] A possible explanation might be that in our study coagulase-negative staphylococci were responsible for 78% of the proven LOS. Only three preterm infants developed a proven LOS caused by gram negative bacteria and one preterm infant a proven meningitis caused by *Escherichia coli*. In our study population, LOS caused by CoNS was not associated with more echo abnormalities in general than other causal agents or clinical infection episodes.

Earlier studies have shown that gram-negative bacteria cause the highest mortality among preterm infants.[9, 21] Our findings support these results; two out of four preterm infants who developed an infection caused by gram-negative bacteria died during the infection episode. The current study also showed that an infection with *Escherichia coli* has the largest impact on the developing brain; two out of three preterm infants developed major brain abnormalities (e.g. ventriculitis and ventricular hemorrhage grade III), which in both cases resulted in dilation of the ventricles. These findings suggest that infection with gram-negative bacteria not only have a large impact on the mortality rates, but may also cause short-term changes of the developing preterm brain. Therefore, cerebral MRI after a proven LOS has to be considered.

An unanticipated finding in our study was that 14% (n=2) of the preterm infants infected with *Staphyloccocus aureus*, developed abnormalities of the thalamus without other major brain abnormalities on cerebral ultrasound.

Hemodynamically significant patent ductus arteriosus (HS-PDA) alone is a known risk factor for major brain abnormalities.[22-24] In the present study we observed that preterm infants with a HS-PDA were more prevalent in the group with only clinical symptoms of LOS. Possibly, a HS-PDA mimics the clinical symptoms of LOS, leading to an overestimation of the number of preterm infants with probable LOS.

Strengths of our study include its prospective element. Because of the prospective design of this study, PCR could be used to differentiate between causal agents found by blood culture. Blood culture is the gold standard for diagnosis of LOS in preterm infants, but adding PCR increases the specificity of bacteria causing LOS by informing us about the specific bacterial load.[25-26] Furthermore, long term follow up will be achieved for all included preterm infants over time which will provide more information about the neurodevelopmental outcome of preterm infants with minor and major brain abnormalities seen on cerebral ultrasound during a sepsis episode.

We also acknowledge several limitations of our study. This is a single center study with a relatively small number of preterm infants that were included in this study. Due to our strict criteria for suspicion of LOS and thus inclusion in our study, most of the preterm infants had a positive blood culture. As a result the reference group without positive blood culture is limited. This, and the rare occurrence of adverse outcome, does not allow statistical modelling. Furthermore, the high incidence of CoNS limits the number of other infections in this study group, leading to a small group of patients with a proven LOS caused by other bacteria. Therefore, it is difficult to assess the prevalence of major brain abnormalities in LOS caused by other bacteria than coagulase-negative staphylococci. Cerebral ultrasound images were analyzed. Not all preterm infants in our study had been subjected to three cerebral ultrasounds (13%). Further studies are needed before the association between LOS and the development of brain abnormalities can be more clearly understood. In fact, to minimize missing data cerebral ultrasound images should have been prospectively gathered the day at onset (day 0), day 3 and day 7 after the onset of (probable) LOS.

In conclusion

This study cerebral ultrasound imaging has shown that only two of the 66 preterm infants with a proven LOS caused by CoNS developed major cerebral abnormalities. These findings might suggest a minor role in at least short-term term effects of coagulase-negative staphylococci in the developing preterm brain. For now follow-up of these preterm infants, has to specify if the short-term outcome of CoNS is a good predictor in the neurodevelopmental outcome further in life.

References

1. Adams-Chapman I, Stoll BJ. Neonatal infection and long-term neurodevelopmental outcome in the preterm infant. Curr Opin Infect Dis. 2006;19(3):290-7.
2. Schlapbach LJ, Aebischer M, Adams M, et al. Impact of sepsis on neurodevelopmental outcome in a Swiss National Cohort of extremely premature infants. Pediatrics. 2011;128(2):e348-e57.
3. Graham EM, Holcroft CJ, Rai KK, et al. Neonatal cerebral white matter injury in preterm infants is associated with culture positive infections and only rarely with metabolic acidosis. Am J Obstet Gynecol. 2004;191(4):1305-10.
4. Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and Growth Impairment Among Extremely Low-Birth-Weight Infants With Neonatal Infection. JAMA. 2004;292(19): 2357-65.
5. Rand KM, Austin NC, Inder TE, et al. Neonatal Infection and Later Neurodevelopmental Risk in the Very Preterm Infant. J Pediatr. 2016;170:97-104.
6. Raymond J, Aujard Y. Nosocomial infecgtions in pediatric patients: a European, multicenter prospective study. European Study Group. Infect Control Hosp Epidemiol. 2000;21(4):260-3.
7. Geffers C, Baerwolff S, Schwab F, et al. Incidence of healthcare-associated infections in high-risk neonates: results from the German surveillance system for very-low-birthweight infants. J Hosp Infect. 2008;68(3):214-21.
8. Bartels DB, Schwab F, Geffers C, et al. Nosocomial infection in small for gestational age newborns with birth weight <1500 g: a multicentre analysis. Arch Dis Child Fetal Neonatal Ed. 2007;92(6):F449-F53.
9. Stoll BJ, Hansen NI, Fanaroff AA, et al. Late-Onset Sepsis in Very Low Birth Weight Neonates: The Experience of the NICHD Neonatal Research Network. Pediatrics. 2002;110(2):285-91.
10. Sarvikivi E, Karki T, Lyytikainen O, et al. Repeated prevalence surveys of healthcare-associated infections in Finnish neonatal intensive care units. J Hosp Infect. 2010;76(2):156-60.

11. van Haastert IC, Groenendaal F, Uiterwaal CS, et al. Decreasing incidence and severity of cerebral palsy in prematurely born children. J Pediatr. 2011;159(1):86-91 e1.
12. Leijser LM, Steggerda SJ, de Bruïne FT, et al. Brain imaging findings in very preterm infants throughout the neonatal period: Part II. Relation with perinatal clinical data. Early Human Development. 2009;85(2):111-5.
13. Brouwer A, Groenendaal F, van Haastert IL, et al. Neurodevelopmental outcome of preterm infants with severe intraventricular hemorrhage and therapy for post-hemorrhagic ventricular dilatation. J Pediatr. 2008;152(5):648-54.
14. Futagi Y, Toribe Y, Ogawa K, et al. Neurodevelopmental outcome in children with intraventricular hemorrhage. Pediatr Neurol. 2006;34(3):219-24.
15. Volpe JJ. Intraventricular hemorrhage in the premature infant-current concepts. Part II. Ann Neurol. 1989;25(2):109-16.
16. de Vries LS, Eken P, Dubowitz LMS. The spectrum of leukomalacia using cranial ultrasound. Behav Brain Res. 1992;49(1):109-16.
17. Kliegman RM, Walsh MC. Neonatal necrotizing enterocolitis: pathogenesis, classification, and spectrum of illness. Curr Probl Pediatr. 1987;14(4):213-88.
18. Shah DK, Doyle LW, Anderson PJ, et al. Adverse neurodevelopment in preterm infants with postnatal sepsis or necrotizing enterocolitis is mediated by white matter abnormalities on magnetic resonance imaging at term. J Pediatr. 2008;153(2):170-5, 5 e1.
19. Glass HC, Bonifacio SL, Chau V, et al. Recurrent postnatal infections are associated with progressive white matter injury in premature infants. Pediatrics. 2008;122(2):299-305.
20. Chau V, Brant R, Poskitt KJ, et al. Postnatal infection is associated with widespread abnormalities of brain development in premature newborns. Pediatr Res. 2012;71(3):274-9.
21. Benjamin DK, DeLong E, Cotten CM, et al. Mortality following blood culture in premature infants: increased with Gram-negative bacteremia and candidemia, but not Gram-positive bacteremia. J Perinatol. 2004;24(3):175-80.
22. Brunner B, Hoeck M, Schermer E, et al. Patent ductus arteriosus, low platelets, cyclooxygenase inhibitors, and intraventricular hemorrhage in very low birth weight preterm infants. J Pediatr. 2013;163(1):23-8.
23. Evans N, Kluckow M. Early ductal shunting and intraventricular haeorrhage in ventilated preterm infants. J Pediatr. 1996;75(3):23-8.
24. Dykes FD, Lazzara A, Ahmann P, et al. Intraventricular hemorrhage: a prospective evaluation of etiopathogenesis. Pediatrics. 1980;66(1):42-9.
25. Lucignano B, Ranno S, Liesenfeld O, et al. Multiplex PCR allows rapid and accurate diagnosis of bloodstream infections in newborns and children with suspected sepsis. J Clin Microbiol. 2011;49(6):2252-8.
26. Paolucci M, Landini MP, Sambri V. How can the microbiologist help in diagnosing neonatal sepsis? Int J Pediatr. 2012;2012:120139.

Chapter 6

Motor outcome in preterm infants with and without nosocomial infection during NICU admittance at six and twelve months corrected age

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Abstract

Background and objective:

Is early gross motor development delayed in preterm infants who experienced late-onset sepsis?

Methods:

Prospective cohort study collecting preterm infants <32 weeks gestational age and/or <1500 grams admitted to the level III Neonatal Intensive Care Unit with suspected late-onset sepsis (LOS).

Results:

In 117 infants one or more episodes of suspicion of LOS were analysed. In 86 infants bacterial culture turned in positive. Twelve infants died during admission, one died after discharge. At six months Alberta Infant Motor Scale (AIMS) scores differed significant between groups with (n=62) or without (n=20) proven LOS (p=0.050) in favour of the LOS group. In the proven LOS group AIMS significantly declined from six to twelve months (p=0.004). A trend in lower AIMS scores at six months was found in a subgroup of infants with necrotizing enterocolitis (p=0.059).

Conclusion:

Using AIMS differences of gross motor development were detected at six months in favor of preterms with late-onset sepsis. At twelve months deterioration was observed, suggesting late-onset sepsis might affect motor development later on. An additional finding is a trend to lower AIMS scores at six months, found in the group of patients with NEC. At twelve months no difference could be found in this subgroup.

Introduction

The incidence of prematurity is approximately 7.7% of all births in the Netherlands. In 1.3% of all births the gestational age is less than 32 weeks.[1] Prematurity is associated with increased mortality and morbidity.[2] This includes somatic problems such as lung disease and retinopathy and also impaired psychomotor development with associated learning disabilities, which is an important outcome parameter of neonatal intensive care treatment.[3]

During admission to the intensive care unit many hazardous episodes will occur, compromising the development of the infants brain and thereby the long term psychomotor outcome. One of the associated risk factors for impaired neurodevelopmental outcome is sepsis.[4, 5] Preterm and low birth weight infants have a high risk of acquiring nosocomial sepsis up to 33% in preterms under 28 weeks gestational age, associated with length of hospital stay and need for invasive devices. Another factor might be an immature immune system.[6, 7]

Dammann et al. described an association of intraventricular hemorrhage or periventricular white matter lesions and pro-inflammatory cytokines like IL-6 due to intra-uterine infection.[8] White matter injury has a role in disturbance of cerebral networking and control and modulation of the motor system and thus on neurodevelopmental outcome.[9]

Long term motor outcome is one of the most important outcome parameters of neonatal intensive care. Mitha et al. reported a higher incidence of cerebral palsy following neonatal sepsis compared to infants without an infectious episode during admittance.[10] Early detection of developmental delay is desirable, to implement early intervention in order to improve long term outcome. Several screening tools are used for assessment of neurodevelopment at different ages.

One of the screening tools for gross motor development in early infancy is the Alberta Infant Motor Scale (AIMS). The AIMS is designed to measure gross motor skills from term equivalent age including independent walking, sit and stand.[11] Components tested in the AIMS are weight bearing properties, posture and antigravity movements.[12] Though this scale was not designed as a predictive tool, it has moderate to excellent predictive validity. It can be used to classify the infants development as normal or suspicious/ abnormal at twelve months corrected age.[13]

The aim of this study is to analyze whether early gross motor development is different in preterm infants who experienced late-onset sepsis during their Neonatal Intensive Care hospitalization compared to infants who did not have a sepsis.

Methods

Prospective cohort study collecting preterm infants <32 weeks gestational age and/or <1500 grams admitted to the level III Neonatal Intensive Care Unit of the VU Medical Center between March 2008 and December 2014 suspected of late-onset sepsis (LOS). Preterms with syndromal or chromosomal abnormalities and congenital metabolic disorders were excluded. Written informed parental consent was obtained and approval was given by the medical ethical committee of the VU University Medical Center.

LOS was suspected when one of the following clinical symptoms occurred: hypothermia (<36.5 °C) or hyperthermia (>37.5 °C) or temperature instability, hypotension, tachycardia, apnea, feeding problems, irritability and/or apathy. Venapuncture and lumbar puncture were performed to obtain cultures. LOS was defined as a positive blood culture after 72 hours of life. In case of coagulase-negative staphylococci (CoNS) an elevated C-reactive protein (CRP) within two days of blood culture was required for a final diagnosis. When blood culture did not turn positive but the clinical signs implied antibiotic treatment for seven days, LOS was considered probable but not proven.

All preterm infants who were suspected for LOS received treatment with intravenously administered amikacin and penicillin G for at least three days. Depending on the causal agent found by blood culture, this combination of antibiotics was continued or was switched to another antibiotic regime. If the blood culture was sterile and CRP was not elevated, antibiotics were discontinued. However, in case of clinical LOS and/or elevated C-reactive protein, antibiotics were continued for seven days.

Clinical data were collected from the patients' medical charts. Neonatal variables included gestational age at birth, birth weight, gender, days of ventilation, >24 hours steroid treatment for either respiratory or circulatory insufficiency, intraventricular hemorrhage according to Papile,[14] lumbar puncture, hemodynamic significant patent ductus arteriosus (HS-PDA), surgery, necrotizing enterocolitis (NEC) stage II or III according to Bell[15] and bronchopulmonary dysplasia grading according to Jobe.[16] Surgical interventions were defined as abdominal, cardiac and ophthalmological surgery, or surgery for other indications.

All surviving patients were invited for the outpatient clinic follow-up at six and twelve months corrected age. During this visit physical examination was performed by a neonatologist, and the Alberta Infant Motor Scale was assessed by a pediatric physiotherapist and expressed in Z-scores. Statistical analysis was performed with SPSS using version 21 (SPSS Inc, Chicago, Illinois, USA). Statistical analyses were performed with Chi-square using Fisher's exact test as appropriate for dichotomous data and T test for paired data. For independent data the independent T test was used. A probability p-value < 0.05 was considered statistically significant.

Results

The patient characteristics are stated in *Table 6.1*. Due to inclusion criteria of gestational age <32 weeks and/or <1500 gram there is a broad range in these variables. Major co-morbidity is stated in *Table 6.2*. In nine patients the indication for surgery was gastro-intestinal

Table 6.1: Patient characteristics (n=117)

Patient characteristics	N (%) or median (range)
Gestational age (wks)	28 0/7 (24 0/7 – 39 5/7)
Birth weight (grams)	1000 (405 – 1965)
Birthweight SDS	0.12 (-5.10 – +3.02)
Head circumference (cm)	25.0 (20.0 – 32.0)
Head circumference SDS	-0.29 (-8.61 – +2.45)
Apgar score 5 min	8 (2 – 10)
Umbilical cord pH	7.28 (6.89 – 7.48)
Chorioamnionitis	29.1%
Male	49.5%
Survival	89.7%

Median (minimum-maximum); wks, weeks; SDS, standard deviation score.

Table 6.2: Major co-morbidity

Morbidity	Incidence (%)
RDS ≥ grade III	37 (31.6)
Need for mechanical ventilation	59 (50.4)
NEC ≥ grade II	20 (17.1)
Need for inotropes	3 (2.6)
IVH ≥ grade II	13 (11.1)
PVL	6 (5.1)
Surgical intervention	10 (8.5)
Corticosteroid use postpartum	19 (16.2)
BPD	23 (19.7)

RDS, Respiratory distress syndrome; NEC, Necrotizing enterocolitis; IVH, Intraventricular Hemorrhage; PVL, Periventricular leukomalacia; BPD, Bronchopulmonary dysplasia.

Table 6.3: Causal infectious agents in blood culture

Causal micro-organism	Incidence (number of cultures)
CoNS	69
Staphylococcus aureus	16
Escherichia coli	2
Klebsiella oxytoca	2
Enterococcus faecalis	1
Streptococcus agalactiae	1
Gram positive rod, not further specified	1

CoNS, Coagulase-negative staphylococcus.

problems, in one for placement of central venous lines. In *Table 6.3* the causal agents in the blood culture are presented. Of the 117 patients, 21 patients had two and one patient had three infectious episodes to be analyzed in this study. If patients had more than one infectious episode the causal agents were stated separately. In 32 of the 117 patients, blood culture revealed no micro-organisms, three of these patients had two clinical infectious episodes in which both blood cultures were negative. Of the 117 patients suspected LOS in total 85 were proven with positive blood cultures, of the 32 patients with negative blood cultures 14 were treated with antibiotics for seven days due to clinical symptoms and in 18 antibiotics were discontinued after 48 hours due to negative blood culture and no clinical suspicion of LOS.

Cerebral fluid was obtained in 68 patients of which 61 samples showed no micro-organisms, in three coagulase-negative staphylococci were found and in four patients *Staphylococci aureus* were isolated.

Of the cohort 12 patients died during admission, one deceased after discharge due to progressive neuromuscular disease.

Table 6.4 presents biometric parameters at birth, six and twelve months corrected age. No differences are seen between the groups with or without proven LOS (resp. n=85 and 32). Nor was a difference in the prevalence of chorioamnionitis between both groups (28.6% vs 31.3%, p=0.821). *Table 6.5* presents gross motor development at six and twelve months corrected age. At six months AIMS Z-score a significant difference in favor of the sepsis group (median Z-score (minimum-maximum) in proven LOS group -0.64 (-2.78 – +0.80) versus -1.14 (-2.29 – +0.80) in the non-proven LOS group, p=0.050). This difference could not be reproduced at twelve months corrected age (median Z-score in proven LOS group -0.89 (-5.22 – +0.66) versus -0.69 (-6.336 – +0.31) in the non-proven LOS group,

Table 6.4: Comparison of birth weight and head circumference at birth, six and twelve months

	Proven LOS (n=85)	No proven LOS (n=32)	p-value
Birth weight	0.11 (-2.39 – +3.02)	-0.14 (-5.10 – +2.80)	0.669
Birth HC	-0.24 (-2.22 – +2.45)	-0.36 (-8.61 – +1.70)	0.252
Gestational age (wks)	28 0/7 (24 0/7-33 4/7)	28 0/7 (24 2/7 – 39 5/7)	0.888
Weight 6 months	-0.28 (-2.87 – +2.60)	-0.69 (-2.70 – +1.98)	0.201
HC 6 months	-0.22 (-2.08 – +2.07)	-0.30 (-2.15 – +1.56)	0.321
Weight 12 months	-0.91 (-2.89 – +2.44)	-1.23 (-3.85 - +1.47)	0.347
HC 12 months	-0.49 (-2.14 – +1.93)	-0.36 (-2.14 – +1.57)	0.952

LOS, late-onset sepsis; HC, head circumference.
Weight and head circumference stated in median standard deviation scores (minimum – maximum), gestational age stated in weeks (median, minimum and maximum), Mann Whitney U test.

Table 6.5: Comparison of AIMS Z-score at six and twelve months corrected age

AIMS Z-score	No of patients assessed	Proven LOS n=85	No of patients assessed	No proven LOS N=32	p-value
6 months	62/85	-0.64 (-2.78 – +0.80)	20/32	-1.14 (-2.29 – +0.80)	0.050
12 months	65/85	-0.89 (-5.22 – +0.66)	20/32	-0.69 (-6.336 – +0.31)	0.378

LOS, late-onset sepsis; AIMS, Alberta Infant Motor Scale, stated in median (minimum-maximum), Mann-Whitney U test.

p=0.378). Differences in AIMS Z-score at six and twelve months were not significant whether meningitis was present or not (p-value resp. 0.763 and 0.160). *Table 6.6* shows the changes in head circumference and AIMS over time.

In our cohort 75% of the positive blood cultures revealed presences of coagulase-negative staphylococci. When dividing the LOS group in LOS with other causal agents than coagulase-negative staphylococci versus non-proven LOS no significant difference could be found (six months p=0.687, twelve months p=0.083). However, at six months, the AIMS between the group with infection by coagulase negative staphylococci (n=50) to the group with late-onset sepsis by other causal agents (n=12) is significant different in favor of the CoNS group (p=0.008). This difference could not be demonstrated at twelve months (p=0.219).

With respect to co-morbidity six month AIMS Z-scores showed a trend to lower scores in patients with necrotizing enterocolitis (*Table 6.7*). In total four patients with NEC did not survive. Surgery was performed in nine patients, in five because of complications of

Table 6.6: Change of head circumference and AIMS between six and twelve months

	HC 6 months	HC 12 months	p-value	AIMS 6 months	AIMS 12 months	p-value
Proven LOS	-0.22 (-2.08 – +2.07)	-0.49 (-2.14 – +1.93)	<0.001	-0.64 (-2.78 – +0.80)	-0.89 (-5.22 – +0.66)	0.004
No proven LOS	-0.30 (-2.15 – +1.56)	-0.36 (-2.14 – +1.57)	0.559	-1.14 (-2.29 – +0.80)	-0.69 (-6.33 – +0.31)	0.642

LOS, late-onset sepsis; HC, head circumference, stated in median standard deviation scores (minimum-maximum); AIMS, Alberta Infant Motor Scale, stated in median (minimum-maximum), Wilcoxon Signed Rank test.

Table 6.7: Comorbidity in neonatal period and AIMS at six months

	Present	Absent	p-value
RDS ≥ grade III	-0.67 (-2.57 – +0.80)	-0.77 (-2.78 – +0.80)	0.940
Need for mechanical ventilation	-0.75 (-2.78 – +0.80)	-0.67 (-2.15 – +0.80)	0.985
NEC ≥ grade II	-1.05 (-2.57 – +0.38)	-0.60 (-2.78 – +0.80)	0.059
IVH ≥ grade II	-0.60 (-1.51 – +0.80)	-0.72 (-2.78 – +0.80)	0.973
PVL I-III	-0.71 (-1.51 – +0.13)	-0.70 (-2.78 – +0.80)	0.876
Surgical intervention	-1.02 (-2.15 – -0.60)	-0.67 (-2.78 – +0.80)	0.120
BPD	-0.78 (-2.29 – +0.31)	-67 (-2.78 – +0.80)	0.876

AIMS, Alberta Infant Motor Scale; RDS, Respiratory distress syndrome; NEC, Necrotizing enterocolitis; IVH, Intraventricular Hemorrhage; PVL, Periventricular leukomalacia; BPD, Bronchopulmonary Dysplasia. AIMS Z score in median (minimum-maximum), Mann-Whitney U test.

NEC, in four because of solitary perforation without signs of NEC. No differences were found between the groups with or without surgery (p=0.120). Two of the nine patients who had surgery did not survive. In 80% (n=16) of the patients with NEC blood culture was positive: in 11 patient CoNS was found and in five other causal agents (*S. aureus*, *Klebsiella* and *E. coli*).

Incidence of other co-morbidity did not reveal any differences between the proven and no proven LOS groups.

Discussion

In our study a significant differences were found in six month neurodevelopmental outcome between the patient groups with or without proven late-onset sepsis was found in favor of the proven infection group, as measured with the AIMS. Though at twelve months infants with a proven LOS deteriorated in neurodevelopmental outcome, whereas infants in the non-proven LOS group did not.

Normal six month outcome after LOS

This might be due to the high incidence of coagulase-negative staphylococci, with their relative mild course of disease during hospitalization. However, studies have shown the adverse effects of these relative mild infections on long-term outcome.[10, 17]

Perhaps six months is too early to screening with AIMS for gross motor developmental delay. On the other hand, Prins et al. reported abnormal AIMS at three and nine months with normal motor outcome at four years, however these children were less preterm then our cohort.[18] Another explanation might be the relatively low sensitivity of the AIMS at six months of age.[19] And finally, the number of infants is skewed in the proven (n=85) and non-proven LOS (n=32) groups.

Deterioration between six and twelve months corrected age

We found a trend in lower AIMS scores at six months in patients with necrotizing enterocolitis, also with lower scores at twelve months although this difference is less clear then in the proven LOS group. The explanation why in infants with NEC the AIMS scores already deviate at six months in comparison to the LOS group might be the result of protracted inflammatory state during the course of the necrotizing enterocolitis,[20] a longer hospitalization period and impaired growth. Another factor might be the effect of anesthesia during surgery. In general, it is presumed general anesthesia might have effects on the development of the preterm brain. One randomized study has been performed showing no differences in neurodevelopmental outcome after surgery with local versus general anesthesia. However, only half of this cohort was born preterm and surgery was performed elective.[21] In our study 25% of the patients with NEC underwent surgery due to a protracted course to recovery, thus the result of the previous study is hardly applicable to our cohort.

Our patients are also in the patient category dependent on central venous catheters for total parenteral nutrition which is an extra risk factor for bloodstream infections.[22]

The patients with no proven LOS had lower birth weight, however not significant, and had a significant smaller head circumference at birth. These biometrics persisted at six months corrected age, however these differences at six months were not significant anymore suggesting catch-up of a possible adverse intra-uterine environment. Neurodevelopment showed a trend to the detriment of the no proven LOS group during the period of six to twelve months corrected age. One could speculate, the adverse intra-uterine environment might have effects on later development as well. In the Netherlands, a specially developed follow up program including physiotherapy is initiated to evaluate neurodevelopment of the NICU population.[23] If a developmental delay is suspected during this follow up period, physiotherapeutic intervention is intensified and if required additional treatment will be commenced. This intensified physiotherapeutic intervention might explain the improvement of neurodevelopment at the corrected age of twelve months.

Limitations

This study also has several limitations. Several infants experienced two or more infectious episodes and only one episode was included in the study, due to ethical or practical causes (for example parents withdrew informed consent during the second infectious episode of an arterial catheter could not be inserted for sampling). This implicates the possibility of more damage done by repeatedly exposure to inflammatory agents in the same patient, whereas only one episode has been registered. Also the presence of chorioamnionitis may have an effect on long term outcome, although in this cohort no difference in prevalence between the proven versus non proven infection groups was demonstrated.

In our cohort a high incidence of coagulase-negative staphylococci is present, in 75% of the positive blood cultures. Other studies report incidences of 40 to 60%.[7, 22] The course of a coagulase-negative staphylococci sepsis tends to be milder than sepsis by other micro-organisms, which might implicate less detrimental effects of the inflammatory response. However delay following coagulase negative staphylococci has been reported.[17]

In conclusion

In this study we detect a difference in early development at six months of age in favor of preterms experiencing late-onset sepsis. However, later follow up at twelve months revealed aberrant neurodevelopment, suggesting late-onset sepsis nevertheless may be a factor in the development of gross motor developmental delay.

Additionally the patients with NEC, seem more at risk for a deterioration of the development, which might be induced by protracted exposure to inflammatory responses and thus earlier presentation of gross motor developmental delay.

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References

1. Schaaf JM, Mol BW, Abu-Hanna A, et al. Trends in preterm birth: singleton and multiple pregnancies in the Netherlands, 2000-2007. BJOG. 2011;118(10):1196-204.
2. Iacobelli S, Combier E, Roussot A, et al. Gestational age and 1-year hospital admission or mortality: a nation-wide population-based study. BMC Pediatr. 2017;17(1):28.
3. Potharst ES, van Wassenaer AG, Houtzager BA, et al. High incidence of multi-domain disabilities in very preterm children at five years of age. J Pediatr. 2011;159(1):79-85.
4. Schlapbach LJ, Aebischer M, Adams M, et al. Impact of sepsis on neurodevelopmental outcome in a Swiss National Cohort of extremely premature infants. Pediatrics. 2011;128(2):e348-e57.
5. Adams-Chapman I, Stoll BJ. Neonatal infection and long-term neurodevelopmental outcome in the preterm infant. Curr Opin Infect Dis. 2006;19(3):290-7.
6. Bartels DB, Schwab F, Geffers C, et al. Nosocomial infection in small for gestational age newborns with birth weight <1500 g: a multicentre analysis. Arch Dis Child Fetal Neonatal Ed. 2007;92(6):F449-F53.
7. Tsai MH, Hsu JF, Chu SM, et al. Incidence, clinical characteristics and risk factors for adverse outcome in neonates with late-onset sepsis. Pediatr Infect Dis J. 2014;33(1):e7-e13.
8. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res. 1997;42(1):1-8.
9. Adams-Chapman I. Insults to the developing brain and impact on neurodevelopmental outcome. J Commun Disord. 2009;42(4):256-62.
10. Mitha A, Foix-L'Helias L, Arnaud C, et al. Neonatal infection and 5-year neurodevelopmental outcome of very preterm infants. Pediatrics. 2013;132(2):e372-e80.
11. Darrah J, Piper M, Watt MJ. Assessment of gross motor skills of at-risk infants: predictive validity of the Alberta Infant Motor Scale. Dev Med Child Neurol. 1998;40(7):485-91.
12. Spittle AJ, Doyle LW, Boyd RN. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. Dev Med Child Neurol. 2008;50(4):254-66.
13. George JM, Boyd RN, Colditz PB, et al. PPREMO: a prospective cohort study of preterm infant brain structure and function to predict neurodevelopmental outcome. BMC Pediatr. 2015;15:123.
14. Papile LA, Burstein J, Burstein R, et al. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978;92(4):529-34.
15. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978;187(1):1-7.
16. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;163(7):1723-9.
17. Alshaikh B, Yee W, Lodha A, et al. Coagulase-negative staphylococcus sepsis in preterm infants and long-term neurodevelopmental outcome. J Perinatol. 2014;34(2):125-9.
18. Prins SA, von Lindern JS, van Dijk S, et al. Motor Development of Premature Infants Born between 32 and 34 Weeks. Int J Pediatr. 2010;2010. pii: 462048.
19. Jeng SF, Yau KI, Chen LC, et al. Alberta infant motor scale: reliability and validity when used on preterm infants in Taiwan. Phys Ther. 2000;80(2):168-78.
20. Adams-Chapman I. Long-term impact of infection on the preterm neonate. Semin Perinatol. 2012;36(6):462-70.
21. Davidson AJ, Disma N, de Graaff JC, et al. Neurodevelopmental outcome at 2 years of age after general anaesthesia and awake-regional anaesthesia in infancy (GAS): an international multicentre, randomised controlled trial. Lancet. 2016;387(10015):239-50.

22. van der Zwet WC, Kaiser AM, van Elburg RM, et al. Nosocomial infections in a Dutch neonatal intensive care unit: surveillance study with definitions for infection specifically adapted for neonates. J Hosp Infect. 2005;61(4):300-11.
23. Koldewijn K, Wolf MJ, van Wassenaer A, et al. The Infant Behavioral Assessment and Intervention Program for very low birth weight infants at 6 months corrected age. J Pediatr. 2009; 154(1):33-8 e2.

Chapter 7

Neurodevelopmental outcome at two years of age in preterm infants with late-onset sepsis

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Abstract

Background and objectives:

Late-onset sepsis is associated with impaired neurodevelopmental outcome in preterm infants.

Methods:

This prospective cohort study aims to establish the effect of sepsis after 72 hours of life on cognitive, psychomotor, and language development of preterm infants (below 32 weeks gestational age and/or below 1500 grams). At two years corrected age, neurodevelopmental outcome was tested using Bayley Scales of Infant Development-II, Lexilijst (lexical development questionnaire) and behaviour checklists.

Results:

Of 117 patients included, 85 experienced blood culture proven infection. Coagulase negative staphylococci were responsible for 55% of the episodes. No significant differences were found in cognitive, motor and behavioural scores or lexi quotient comparing patients with versus no proven infection. When comparing three groups (coagulase negative staphylococci, other and negative blood culture) a significant difference was found in composite cognitive scores ($p=0.016$), in favor of the coagulase negative staphylococci group versus other causal agents group ($p=0.007$). No significant differences were found in other subscales.

Conclusion:

In this cohort no differences were found in neurodevelopmental outcome at two years corrected age between proven versus no proven infection group, confirmation in larger cohorts with a control group is needed. Patients encountering coagulase negative staphylococci sepsis showed significant better cognitive outcome compared to other causal agents.

Introduction

Long term neurodevelopmental outcome is one of the most important outcome parameters of neonatal intensive care. The long term developmental outcome is, however, at risk due to the hazardous events that will occur during admission to the neonatal intensive care unit (NICU). These events are potentially dangerous for the brain development of the infants and may inflict delay in mental and/or psychomotor development. Examples of these events are respiratory or circulatory insufficiency, intracranial hemorrhage or resuscitation

Another associated risk factor for impaired neurodevelopmental outcome is sepsis.[1, 2] Preterm and low birth weight infants have a high risk of acquiring late-onset sepsis during their admission to the NICU. The risk of late-onset sepsis is ranging from 33% in preterms with a gestational age less than 28 weeks to 60% in preterms less than 25 weeks.[3] Late-onset sepsis is also associated with prolonged hospital stay, length of invasive ventilation and need for invasive devices and parenteral nutrition.[3]

The adverse effects of late-onset sepsis might be due to the inflammatory response and effects of this response on the vulnerable developing brain. Damman et al described an association of intraventricular hemorrhage or periventricular white matter lesions and pro-inflammatory cytokines like IL-6 due to intra-uterine infection.[4] A similar mechanism might occur during postnatal infections. White matter injury has a negative impact on cerebral networking and control, and modulation of the motor system and thus on neurodevelopmental outcome.[5] Mitha et al. reported a higher incidence of cerebral palsy (CP) following neonatal sepsis compared to infants without an infectious episode during admittance.[6]

In early postnatal period a tremendous change in brain structure and function is demonstrated,[7] which makes the brain vulnerable. Studies showed reduced brain volumes in infants after experiencing sepsis,[8] An important factor accounting for favorable long term outcome in preterm infants is adequate weight gain and growth of head circumference. Full enteral nutrition provides better weight gain and less postnatal growth restriction.[9] Infection may have impact on adequate weight gain in several ways. Due to infection enteral nutrition might be less tolerated, leading to cumulative protein and energy deficits, with impaired weight gain. Also, as a consequence, due to longer parenteral nutrition supplementation and the need for central venous lines, the risk to experience a blood stream infection increases.[10]

The aim of this study is to analyze whether neurodevelopmental outcome at two years corrected age is compromised in preterm infants with suspected late-onset sepsis, comparing infants with proven infection to infants without a proven infection, in particular if certain species of causal micro-organism influence long term outcome.

Methods

A prospective cohort study was performed collecting data from preterm infants born with a gestational age <32 weeks and/or <1500 grams admitted to the level III Neonatal Intensive Care Unit of the VU Medical Center between March 2008 and December 2014 suspected of late-onset sepsis. Preterm infants with syndromal or chromosomal abnormalities and congenital metabolic disorders were excluded. The medical ethical committee of the VU University Medical Center approved the study (protocol number 2008/77) and written informed parental consent was obtained during the first days of admittance at the NICU. Patients were included in the study when a suspicion of late-onset sepsis occurred.

Late-onset sepsis was suspected when one of the following clinical symptoms occurred: hypothermia (<36.5 °C) or hyperthermia (>37.5 °C), hypotension, tachycardia, apnea, feeding problems, irritability and/or apathy. Late-onset sepsis was defined as a positive blood culture after 72 hours of life.[11] If the blood culture did not turn positive but clinical signs implied antibiotic treatment for seven days, late-onset sepsis was considered probable but not proven. If in one of the episodes a causal micro-organism was found, the infant was classified as proven infection.

Clinical data were collected from the patients' medical charts. Patient variables included gestational age at birth, birth weight, gender, days of ventilation, >24 hours steroid treatment for either respiratory or circulatory insufficiency, intraventricular hemorrhage (IVH) and periventricular leucomalacia (PVL) according to Volpe,[12] lumbar puncture, hemodynamic significant patent ductus arteriosus (HS-PDA), surgery, necrotizing enterocolitis (NEC) stage II or III according to Bell[13] and bronchopulmonary dysplasia (BPD) according to Jobe.[14] Surgical interventions were defined as abdominal, cardiac and ophthalmological surgery, or surgery for other indications. Positive blood cultures were divided in two groups: coagulase negative staphylococci and other micro-organisms for subanalyses.

All surviving patients were invited for outpatient clinic follow-up at term age, and at 3, 6, 12 and 24 months corrected age. During this visit physical examination was performed, including biometrics. At two years corrected age neurodevelopment was assessed according to the Bayley Scales of Infant and Toddler Development II (BSID-II),[15, 16] lexi quotient[17, 18] and Child Behaviour Checklist (CBCL)[19] by trained pediatric psychologists, who were aware of participants to the study, though unaware of in which study group the patients belonged. Maternal education level was registered. Statistical analysis was performed with SPSS using version 22 (SPSS Inc, Chicago, Illinois, USA). Statistical analyses were performed with Chi-square using Fisher's exact test as appropriate for dichotomous data and T test for paired data. For analysis of two or more groups and confounding, multivariate linear regression techniques were used. Possible confounders were need for invasive ventilation, need for inotropics, postnatal corticosteroids, PVL, NEC or BPD. Confounding was defined as change in regression coefficient over 10%. A probability p-value < 0.05 was considered statistically significant.

Results

Patient enrollment and inclusion is demonstrated in *Figure 7.1* and patient characteristics are described in *Table 7.1*. During the inclusion period 651 patients would have been eligible for inclusion, of which 117 patients were included during an episode of suspicion of infection. Ninety-one patients did not experience an episode of suspicion of infection, and therefore not included in the study. Of the 117 patients with clinical suspicion of late-onset sepsis 19 patients had two episodes (of which in six patients both blood cultures turned positive, in ten patients one blood culture turned positive and in three none of the blood cultures turned positive) and one had three episodes (of which one with positive blood culture) of suspicion of late-onset sepsis.

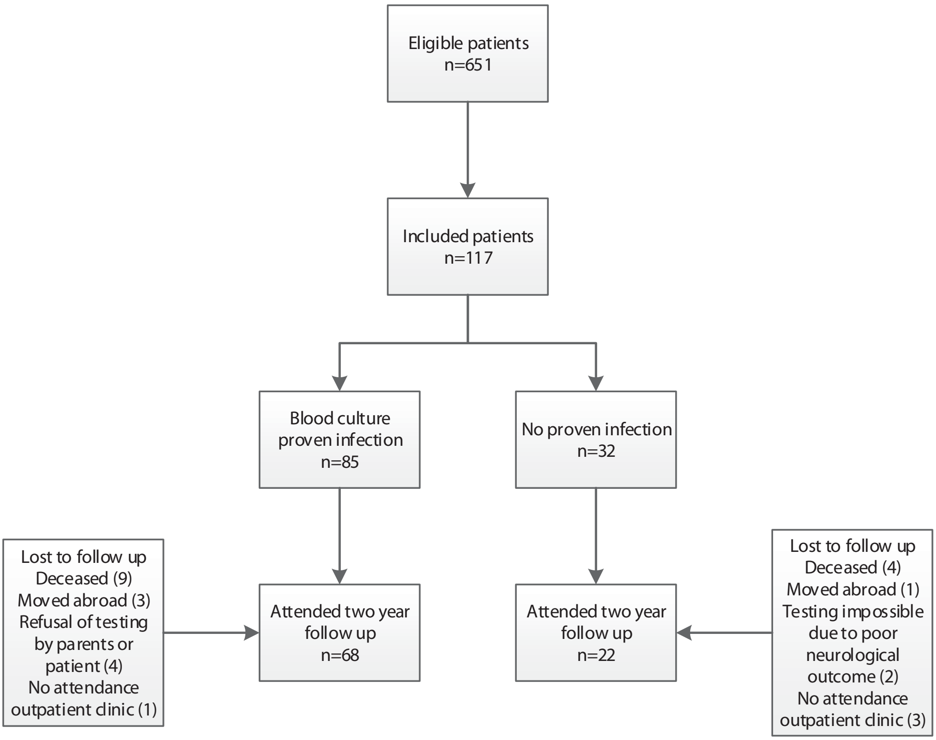

Figure 7.1: Patient inclusion.

Table 7.1: Patient characteristics for suspected late-onset sepsis

	Total (n=117)	Proven LOS (n=85)	No proven LOS (n=32)	p-value
Gestational age (wks, SD in days)	28 1/7 (16 days)	28 0/7 (15 days)	28 2/7 (19 days)	0.713
Gestational age <27 0/7 weeks (n)	33	24	9	>0.999
Gestational age 27 0/7 – 28 5/7 weeks (n)	44	31	13	0.675
Gestational age ≥29 0/7 weeks (n)	40	30	10	0.827
Birth weight (grams)	1061 (330)	1078 (322)	1016 (350)	0.364
Birthweight SDS	0.08 (1.21)	0.16 (1.02)	-0.14 (1.61)	0.330
SGA (SDS <2 SDS)	5	2	3	0.125
Apgar score 5 min	7.4 (1.75)	7,3 (1.9)	7.7 (1.4)	0.190
Umbilical cord pH, arterial	7,27 (0.11)	7.27 (0.11)	7.27 (1.37)	0.936
Male	49.6%	50.6%	46.9%	0.863
Survival	89.7%	89.4%	87.5%	0.259

Mean (SD, standard deviation); LOS, late-onset sepsis; wks, weeks; SGA, small for gestational age; SDS, standard deviation score; T test and Fisher exact test.

In *Table 7.2* the causal agents in the blood culture are described. If patients had more than one infectious episode the causal agents were stated separately. In 32 of the 117 patients, blood culture revealed no micro-organisms, three of these patients had two clinical infectious episodes in which both blood cultures were negative. Of the 117 patients suspected of late-onset sepsis 85 (73%) were proven with positive blood culture. Of the 32 patient with negative blood cultures 14 patients (12%) were treated with antibiotics for seven days due to persistent clinical symptoms and in 18 patients (15%) antibiotics were discontinued after 48 hours due to negative blood culture and recovered of clinical symptoms for suspicion of late-onset sepsis.

In 68 patients cerebral fluid could be obtained for culture. In 61 patients, culture of cerebral fluid showed no micro-organisms. In three coagulase-negative staphylococci and in four patients *Staphylococcus aureus* were isolated. No significant differences could be found in neurodevelopment between patients with proven meningitis versus no meningitis.

In *Table 7.3* significant comorbidity is stated. No significant differences were found in co-morbidity between groups with or without proven infection.

Table 7.2: Causal infectious agents in blood culture

Causal micro-organism	Incidence (number of cultures)
Coagulase-negative staphylococci	70
Staphylococcus aureus	14
Escherichia coli	2
Klebsiella oxytoca	2
Enterococcus faecalis	1
Streptococcus agalactiae	1
Gram positive rod, not further specified	1

Table 7.3: Major co-morbidity

Morbidity	Total incidence n (%)	Proven LOS (n=85)	No proven LOS (n=32)	p-value
RDS ≥ grade III	37 (31.7)	24 (28.2)	13 (40.6)	0.265
Need for mechanical ventilation	59 (50.4)	42 (49.4)	17 (53.1)	0.836
NEC ≥ grade II	20 (17.1)	16 (18.8)	4 (12.5)	0.584
Need for inotropes	3 (2.6)	3 (3.5)	0 (0)	0.561
IVH ≥ grade II	13 (11.1)	9 (10.6)	4 (12.5)	0.749
PVL	6 (5.1)	4 (4.7)	2 (6.3)	0.664
Surgical intervention	9 (7.7)	7 (8.2)	2 (6.3)	>0.999
Corticosteroid use postpartum	19 (16.4)	14 (16.5)	5 (15.6)	>0.999
BPD	23 (19.7)	14 (16.5)	9 (28.1)	0.193

LOS, late-onset sepsis; RDS, Respiratory distress syndrome; NEC, Necrotizing enterocolitis; IVH, Intraventricular Hemorrhage; PVL, Periventricular leukomalacia; BPD, Bronchopulmonary dysplasia, Fisher exact test.

Table 7.4 shows the results of the BSID-II, lexi quotient and Child Behaviour Checklist at two years corrected age. Of the cohort of 117 patients 90 patients were included in the analyses for long term follow up, of which 68 patients in the proven and 22 patients in the non-proven infection group. Reasons for lost to follow up were deceased (13, of which 12 during NICU admission and one after NICU discharge), moved abroad (4), refusal for neurodevelopmental testing by parents or patient (4), impossible testing due to poor neurodevelopmental outcome (one due to cerebral palsy and one due to a cortical migration disorder) and no attendance to outpatient clinic (4).

In the proven late-onset sepsis group 68 patients were tested (80%), in the non-proven late-onset sepsis group 22 (69%). No statistical significant differences were found in cognitive, motor and behavioural scores or in lexi quotient indicating comparable

Table 7.4: Comparison of BSID-II, lexilijst and child behavioural scores at two years of corrected age

	Proven LOS (n=68)	No proven LOS (n=22)	p-value
Corrected age at testing	24m 15d (47d)	24m 12d (28d)	0.759
Composite cognitive score (BSID-II)	100 (9.0)	98 (13.90)	0.276
Composite motor score (BSID-II)	100 (9.4)	99 (12.3)	0.687
Lexi quotient (Lexilijst)	91 (16.1)	88 (18.2)	0.489
Total behavioural score (CBCL)	26 (14.9)	30 (21.2)	0.283
Total internalizing score (CBCL)	5 (4.3)	8 (7.9)	0.171
Total externalizing score (CBCL)	12 (7.5)	12 (7.6)	0.908

Mean (standard deviation). LOS, late-onset sepsis; m, months; d, days. T test.

neurodevelopmental outcomes in both groups. Also, when comparing the proven late-onset sepsis group (68 patients tested, 80%) to the group in which antibiotics were discontinued after 48 hours (12 patients tested, 38%) no significant differences could be demonstrated in either of these neurodevelopmental outcome scores.

In *Table 7.5* the effects of infection on the different domains are presented. Confounding analyses revealed PVL and BPD as confounding factors. When correcting for those confounders (PVL and BPD) the effects decrease substantially, except the effect on behavioural score.

Maternal highest educational level was also taken into account. No differences in maternal education between the groups were found (p=0.381). In multivariate analyses no significant difference could be shown of maternal education.

The group experiencing late-onset sepsis had a trend to a higher risk on poor outcome, defined as BSID-II composite cognitive and/or motor score <-1 SD of general population scores, or dead (p=0.068).

Early markers of possible brain damage, e.g. echodensities on cerebral ultrasound were not associated with differences in the different domains of neurodevelopment (cognitive, motor, and behavioural scores or lexi quotient). Also when comparing the major ultrasound abnormalities no significant differences could be demonstrated.

Due to the high representation of coagulase negative staphylococci (55 patients of the follow group), with a possible milder clinical course and long term effects, the study population has been divided in three groups: coagulase negative staphylococci, other and no proven infection. A significant difference was found in the composite cognitive score subscale

Table 7.5: Effects on neurodevelopment no proven versus proven late-onset sepsis

	Regression coefficient	95% Confidence interval	p-value
Composite cognitive score, crude	2.567	-2.090 – +7.223	0.276
Composite cognitive score, adjusted*	1.892	-2.871 – +6.655	0.432
Composite motor score, crude	1.022	-4.003 – +6.047	0.687
Composite motor score, adjusted*	0.746	-4.452 – +5.943	0.776
Lexi quotient, crude	3.520	-6.605 – +13.644	0.489
Lexi quotient, adjusted*	0.871	-9.038 – +10.780	0.861
Total behavioural score, crude	-4.776	-13.564 – +4.012	0.283
Total behavioural score, adjusted*	-5.139	-16.019 – +3.672	0.241

* Adjusted for confounding by bronchopulmonary dysplasia and periventricular leukomalacia. Linear regression.

(p=0.016). Subanalyses show the difference in favor of the coagulase negative staphylococci group vs the other causal infectious agents group (p=0.007), see *Figure 7.2*. No significant differences were found in the other subscales (composite motor score, lexi quotient and behavioural scores) between the three groups.

Discussion

Long term neurodevelopmental outcomes after admission at the NICU for prematurity is at risk. One of the potential risk factors is late-onset sepsis. Therefore, the present study investigated the effects on long term psychomotor outcome in preterm infants suspected of late-onset sepsis.

No differences in neurodevelopmental outcome at two years corrected age could be demonstrated between groups with or without proven infection. One could suggest this last group might have been going through serious other co-morbidity e.g. intracerebral pathology, protracted respiratory support for which postnatal corticosteroids were administered, and therefore demonstrate impaired neurodevelopmental outcome. However, these differences in co-morbidity in both subgroups could not be demonstrated as shown in *Table 7.3*.

Coagulase negative staphylococci mono-infection represented more than half of all episodes of late-onset sepsis, in 65 patients (55%). The other four had a second episode with a positive blood culture with another microorganism. In literature high incidences of coagulase negative staphylococci are reported.[3] Conflicting results of coagulase negative

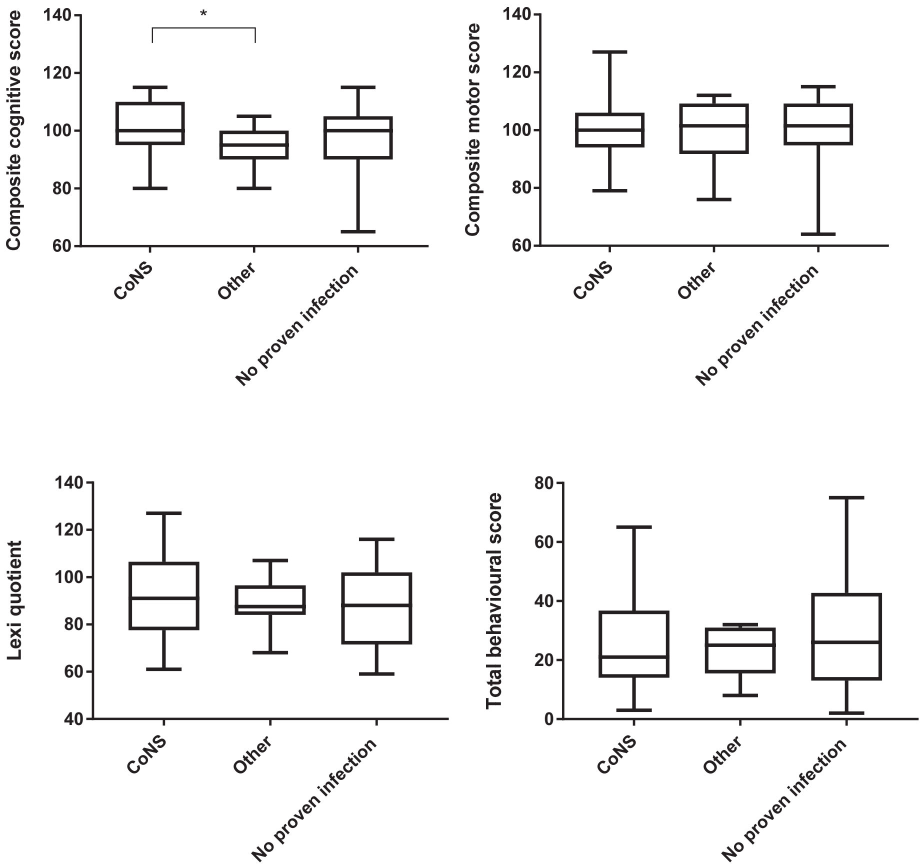

Figure 7.2: Comparison of BSIDII, lexilijst and child behavioural scores at two years of age of CoNS, other infections and no proven infection groups (* p=0.007; T test, CoNS: coagulase negative staphylococci).

staphylococci infections have been reported in previous studies. Alshaikh et al. state coagulase negative staphylococci is associated with increased risk for cognitive delay and major disability.[20] In contradiction, Mittendorf et al. state these infections are not associated with poor neurodevelopmental outcome.[21] Previous studies in infants with coagulase negative staphylococci infections showed a low C-reactive protein (CRP) in approximately one third of the episodes.[22] Possibly, the inflammatory cascade is less activated. This might suggest a lower inflammatory response and possibly less detrimental effects on long term neurodevelopmental outcome.

In our study we demonstrated a significant difference for composite cognitive score in favor of patients experiencing late-onset sepsis caused by coagulase negative staphylococci in comparison to other causative agents. However it is important to note that the majority of this cohort attending follow-up experienced coagulase negative staphylococci sepsis (55/90). Thirteen patients attending follow-up had a causative agent other than coagulase negative staphylococci. No differences were demonstrated in the other domains. Long term cognitive outcome is also known to be influenced by maternal education.[23] However, no significant effect of maternal education level could be demonstrated. In future studies the influence of these socio-economic effects should be studied more extensively.

In an earlier publication cerebral ultrasound abnormalities were explored as an early indicator for adverse neurodevelopmental outcome. In line with the relatively good prognosis of coagulase negative staphylococci infections we did not find major brain damage in this cohort using cerebral ultrasound.[24] This finding is in consistency with the normal scores on the BSID-II at two years of age, corrected for prematurity.

The present study has some limitations. Due to the high representation of coagulase negative staphylococci the effect of other infectious agents are underexposed. On the other hand, the high incidence of coagulase negative staphylococci is common practice in level III NICU's and thus representative for this patient group.

The control group consisting of patients without proven infection is relatively small in comparison to the proven infection group. In addition, the group without proven infection can contain patients with an infection, but not have a positive blood culture. This might influence the neurodevelopmental outcome negatively in the control group and therefore, differences between groups become less. However, no statistical different neurodevelopmental outcome could be demonstrated in subanalysis comparing the proven infection group to the group in which antibiotics were discontinued after 48 hours. One could also argue the load of infectious agents is lower, and possibly the inflammatory cascade is less activated as mentioned before. Future research into inflammatory cytokines and their role in the inflammatory cascade and subsequent effect on neurodevelopmental outcome might give further insights. Also a larger cohort with a substantial control group, and considerable long term follow-up,[25] might elucidate possible effects of inflammation in patients suspected of late-onset sepsis with negative blood culture. Though a control group with preterm infants without any sign of infection, will be challenging. Surrogate markers as CRP or procalcitonin might be needed to construct a "control group".[26]

In conclusion

In this study in preterm born infants no differences in neurodevelopmental outcome at two years corrected age could be demonstrated between the groups with or without proven infection on neurodevelopmental outcome at two years corrected age in preterm infants.

This finding needs replication in larger cohorts with a substantial control group. Patients experiencing coagulase negative staphylococci sepsis have a significantly better cognitive outcome in comparison to other causal infectious agents.

References

1. Schlapbach LJ, Aebischer M, Adams M, et al. Impact of sepsis on neurodevelopmental outcome in a Swiss National Cohort of extremely premature infants. Pediatrics. 2011;128(2):e348-e57.
2. Adams-Chapman I, Stoll BJ. Neonatal infection and long-term neurodevelopmental outcome in the preterm infant. Curr Opin Infect Dis. 2006;19(3):290-7.
3. Boghossian NS, Page GP, Bell EF, et al. Late-onset sepsis in very low birth weight infants from singleton and multiple-gestation births. J Pediatr. 2013;162(6):1120-4, 4 e1.
4. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res. 1997;42(1):1-8.
5. Adams-Chapman I. Insults to the developing brain and impact on neurodevelopmental outcome. J Commun Disord. 2009;42(4):256-62.
6. Mitha A, Foix-L'Helias L, Arnaud C, et al. Neonatal infection and 5-year neurodevelopmental outcome of very preterm infants. Pediatrics. 2013;132(2):e372-e80.
7. Tau GZ, Peterson BS. Normal development of brain circuits. Neuropsychopharmacology. 2010; 35(1):147-68.
8. Bouyssi-Kobar M, du Plessis AJ, McCarter R, et al. Third Trimester Brain Growth in Preterm Infants Compared With In Utero Healthy Fetuses. Pediatrics. 2016;138(5):e20161640.
9. Karagol BS, Zenciroglu A, Okumus N, et al. Randomized controlled trial of slow vs rapid enteral feeding advancements on the clinical outcomes of preterm infants with birth weight 750-1250 g. JPEN J Parenter Enteral Nutr. 2013;37(2):223-8.
10. Mobley RE, Bizzarro MJ. Central line-associated bloodstream infections in the NICU: Successes and controversies in the quest for zero. Semin Perinatol. 2017;41(3):166-74.
11. van der Zwet WC, Kaiser AM, van Elburg RM, et al. Nosocomial infections in a Dutch neonatal intensive care unit: surveillance study with definitions for infection specifically adapted for neonates. J Hosp Infect. 2005;61(4):300-11.
12. Volpe JJ. Neurology of the Newborn. Philedelphia: Saunders Elsevier; 2008.
13. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978;187(1):1-7.
14. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2001;163(7): 1723-9.
15. Bayley N. Bayley Scales of Infant Development. Second Edition. San Antonio1993.
16. Meulen van der BF, Ruiter SAJ, Lutje Spelberg HC, et al. BSID-II-NL. Bayley Scales of Infant Development-II - Nederlandse versie. Amsterdam: Harcourt Test Publishers; 2004.
17. Schlichting JEPT, Spelberg HC. Lexilijst Nederlands. Een instrument om de taalontwikkeling te onderzoeken bij Nederlandstalige kinderen van 15-27 maanden in het kader van vroegtijdige onderkenning. Amsterdam: Pearson Assessment & Information B.V.; 2002.
18. Schlichting JEPT. Lexiconlijsten Marokkaans-Arabisch, Tarifit-Berbers en Turks. Instrumenten om de taalontwikkeling te onderzoeken bij jonge Marokaanse en Turkse kinderen in Nederland. Handleiding. Amsterdam: JIP-Uitgeverij; 2006.
19. Achenbach TM, Rescorla LA. Manual for the ASEBA preschool forms and profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth & Families; 2000.
20. Alshaikh B, Yee W, Lodha A, et al. Coagulase-negative staphylococcus sepsis in preterm infants and long-term neurodevelopmental outcome. J Perinatol. 2014;34(2):125-9.

21. Mittendorf R, Covert R, Kohn J, et al. The Association of Coagulase-Negative Staphylococci Isolated from the Chorioamnion at Delivery and Subsequent Development of Cerebral Palsy. J Perinatol. 2001;1:3-8.
22. Lai MY, Tsai MH, Lee CW, et al. Characteristics of neonates with culture-proven bloodstream infection who have low levels of C-reactive protein (<==10 mg/L). BMC Infect Dis. 2015;15:320.
23. Linsell L, Johnson S, Wolke D, et al. Cognitive trajectories from infancy to early adulthood following birth before 26 weeks of gestation: a prospective, population based cohort study. Arch Dis Child. 2017:1-8.
24. Claessens LC, Zonnenberg IA, van den Dungen FA, et al. Cerebral ultrasound abnormalities in preterm infants caused by late-onset sepsis. PLoS One. 2017;12(3):e0173227.
25. Pierrat V, Marchand-Martin L, Arnaud C, et al. Neurodevelopmental outcome at 2 years for preterm children born at 22 to 34 weeks' gestation in France in 2011: EPIPAGE-2 cohort study. BMJ. 2017;358:j3448.
26. Sharma D, Farahbakhsh N, Shastri S, et al. Biomarkers for diagnosis of neonatal sepsis: a literature review. J Matern Fetal Neonatal Med. 2018;31(12):1646-59.

Chapter 8

General discussion and future perspectives

The general aim of this thesis was to identify predictors for long term neurodevelopmental outcome in term and preterm born infants after perinatal and neonatal events. Therefore different modalities (e.g. cerebral ultrasound, Near InfraRed Spectroscopy and MRI) to predict long term neurodevelopmental outcome in newborns were studied.

Part 1: Perinatal predictors

In term born infants complications during birth are important risk factors for impaired neurodevelopmental outcome. Acute blood loss of the newborn infant is one of these complications. Many events could inflict anaemia, e.g. foetomaternal hemorrhage and exsanguination due to hemorrhage from the placenta or vasa praevia. Less frequent causes are umbilical cord rupture or acute twin-to-twin transfusion. Due to an acute decrease of circulating blood volume and thereby oxygen delivery, cerebral hypoxia may occur.

Mechanisms inducing cerebral damage have been studied mainly in term infants experiencing perinatal asphyxia. Due to brain maturation and increased metabolic demands, the deep grey matter nuclei and central cortex are likely to be affected during an acute hypoxic-ischaemic insult.[1] However, also other injury patterns have been described in term infants,[2] including white matter injury, which is considered to be due to more prolonged and repetitive hypoxic-ischaemic events.

Many studies have been performed investigating effects of perinatal asphyxia due to several underlying causes, however, the component inflicted by acute anaemia has not been addressed before. In a retrospective study (*chapter 2*) we found white matter abnormalities on MRI images of the brain in infants who suffered from acute anaemia as the main pattern of brain injury. Our data show, that if the perinatal asphyxia component is limited and grey matter injury is absent, the long term neurodevelopmental outcome of these infants appears to be more favourable. Next to the effect on motor outcome, it is important to determine the implications of acute neonatal anaemia on higher cognitive functions, which will become increasingly important when these children attend school. If more information about these long term outcome parameters become available by future research, indicating that the favourable development persists at school age, parents can be conciliated with a favourable prognosis if acute anaemia occurs without an evident component of perinatal asphyxia.

Stages and Ages Questionnaires (ASQ) has shown to be a useful method for screening purposes in a larger cohort over a longer period of time. Moreover, this parent-based questionnaire requires minimal effort and time of parents. A gross impression can be obtained of the neurodevelopment addressing five different neurodevelopment domains: communication, gross motor skills, fine motor skills, problem solving capacity and social emotional development. The questionnaire can be used to screen subsequent ages and thus can longitudinally follow the development of children. In *chapter 3* we used the ASQ screening tool to compare a historical cohort experiencing perinatal asphyxia. Therapeutic hypothermia has been implemented sequentially on all NICU's in the Netherlands. A facilitated comparison of an intervention as therapeutic hypothermia in the Netherlands, is at present time impossible since for years therapeutic hypothermia is standard of care for perinatal asphyxia. Even in this small cohort studied, a better long term outcome in the hypothermia group was shown, supporting the low number needed to treat for therapeutic hypothermia.[3]

In the Netherlands, therapeutic hypothermia following perinatal asphyxia is considered in infants over 36 0/7 weeks gestational age. In several studies minimal gestational age criteria for therapeutic hypothermia was set on 34 or 35 weeks gestational age.[3, 4] It would be interesting to investigate whether this favourable effect of hypothermia is also present in preterm infants with a lower gestational age as well. On the other hand, it is known that hypothermia at admission in preterm infant under 28 weeks gestational age is associated with increased morbidity and mortality.[5] To investigate at what gestational age one finds the turning point from harm to benefit for inducing therapeutic hypothermia in infants with perinatal asphyxia would be of great interest.

Part 2: Postnatal predictors

Postnatal risk factors for impaired neurodevelopmental outcome in term and preterm infants are various. However, the implications of these risk factors might be dependent on gestational age. In this part of the thesis we investigated the early and late effects of late-onset sepsis in a cohort of preterm infants.

We hypothesized that a possible disturbance in cerebral autoregulation might occur during late-onset sepsis. Cerebral autoregulation can be monitored by using Near Infrared Spectroscopy (NIRS). In *chapter 4* we monitored a cohort of preterm infants during the first 72 hours of late-onset sepsis by NIRS. This was a subgroup of a larger group of children (n=117).

In the whole cohort and also in the NIRS cohort coagulase staphylococcus (CoNS) was the main causal micro-organism. In literature reference values for regional cerebral oxygenation (rScO2) and relative cerebral fractional oxygen extraction (cFTOE) have been published for the first three days of life.[6] However, comparing our data to these best available reference values no significant differences for rScO2 and cFTOE could be demonstrated during the episode of late-onset sepsis. When looking at neurodevelopmental outcome of these infants at two years corrected age using Bayley Scales of Infant and Toddler Development (BSID-II), version II, no adverse development was demonstrated compared to the general population.

Another hypothesis of the pathogenesis of adverse outcome after late-onset sepsis is the release of inflammatory mediators like interleukines, which can induce and increase cerebral damage, particularly in the white matter.[7, 8] The adverse long term effects of sepsis are linked to the development of white matter injury.[9, 10] With cerebral ultrasound serial imaging can be performed and used to observe changes in the white matter. *Chapter 5* describes the cerebral ultrasound findings in a study population with (suspicion of) late-onset sepsis. We found no differences in major brain abnormalities, e.g. large intra-ventricular hemorrhage, post-hemorrhagic ventricular dilatation or periventricular leukomalacia over grade I. Mild white matter abnormalities were present in one third of all patients, irrespective the presence of a late-onset sepsis. The main causal micro-organism found in this cohort was CoNS. Possibly, the effect of late-onset sepsis by CoNS detected by cerebral ultrasound is less detrimental.

For the analysis of neurodevelopment, several diagnostic tools are available to evaluate the development at various postnatal ages. During infancy neurodevelopment is mostly based on evaluating milestones in motor development. The Alberta Infant Motor Scale (AIMS) is a tool evaluating gross skills development in the first year of life (from term to independent walking). In our cohort at six months corrected age a difference in favor of the sepsis group was found. However, later follow-up at twelve months corrected age revealed aberrant neurodevelopment, suggesting that late-onset sepsis nevertheless might be a factor in the development of gross motor developmental delay. Also, in a subgroup of infants who experienced necrotizing enterocolitis lower neurodevelopmental scores were found.

Another tool is BSID-II (12 to 30 months). This tool enables a more detailed report of neurodevelopment, both on cognitive and motor development. Using this more detailed neurodevelopment assesment to compare patients with and without proven infection, no significant differences in cognitive, motor and behavioural scores of lexi quotient were shown. In this cohort CoNS were responsible for 55% of the episodes. A possible explanation might be that CoNS infections have a relatively mild clinical course and thereby might have less detrimental effect on the developing preterm brain. This was also demonstrated in sub-analyses comparing the infants experiencing CoNS sepsis versus other causal agents. Cognitive outcome scores were significantly higher in the former group. However, in literature contradictory findings on long term neurodevelopmental outcome in infants with late-onset sepsis with CoNS have been reported.[11, 12] The pathophysiology and impact of late-onset sepsis on the neonatal brain has by far not been elucidated yet.

Future perspectives

Many aspects of neonatal risk factors are still unknown and thereby the impact on long term outcome of these infants. The effect of intra-uterine inflammation has been subject of several studies. But what role do inflammatory mediators have on the developing neonatal brain during postnatal sepsis? Moreover, are the effects different in term compared to preterm born infants? In the perspective of the rapidly developing brain during gestation, one can imagine these inflammatory mediators may interfere with normal development of the brain.

Several studies have been performed in animal models. The effect of intrauterine lipopolysaccharides, induced by maternal inflammation, on white matter injury and microglial activation in cerebral white matter tracts with cerebellar injury has been shown in foetal rhesus macaques and neonatal rabbits.[13, 14] Systemic inflammation in preterm born infants activates a cascade of pro-inflammatory cytokines, which in turn leads to activation of microglia in combination with free radical formation inducing maturation dependent cell death and apoptosis of neural cells.[15] Cytokines which may have this effect are IL-6, IL-1β and TNF-α. The possible detrimental effects of IL-6 and IL-1β have already been reported two decades ago.[16] The effects of cytokines could be mediated by cyclooxygenases (COX's) located in the blood brain barrier. COX's are stimulated in inflammatory processes in brain tissue and COX-1 and COX-2 may have a role in microglial activation. On the other hand, COX-2 activity seems to mediate neuroprotection via specific anti-inflammatory lipid mediators as well.[17] This dual role of inflammatory mediators has still not been elucidated.

Also genetic factors may play a role in the development of neurological sequelae in preterm infants. For instance, why does periventricular leukomalacia (PVL) develops only in a small proportion of the admitted infants while most of them experience the same risk factors during hospitalization? Recent studies did find a relation in children with cerebral palsy and de novo genomic copy-number variations.[18]

It is thought, the intrauterine environment might influence and change epigenetic gene regulation. Intra-uterine inflammation itself is associated with postnatal inflammatory response due to epigenetic modifications in Toll like receptors (TLR). TLRs are key elements in the innate immune response.[19] This might induce inflammation, impair the immune system and cause pathologic conditions in the preterm infant.[20] In recent years TLRs become more and more subject of interest. As mentioned, TLRs are pattern recognitions receptors with a key role in the innate immune response. They are activated by pathogen-associated molecular patterns on the surface of bacteria and viruses, leading to the activation of genes encoding pro-inflammatory factors.

The effects of epigenetic- and immunomodulatory factors and their role during postnatal sepsis are still subject of research. This might elucidate the pathophysiology of white matter injury, and hopefully provides possibilities to prevent this white matter injury in the future.

As mentioned before, the neuro-ontogenetic process is a continuing process throughout gestation. Functional networks change enormously during development. One could imagine this development can be influenced by hazardous events during NICU hospitalization. A relation between disturbance in network formation and neuropsychiatric disorders have been reported.[21] To investigate cerebral networks, the measurement of functional cerebral connectivity by electroencephalography can be used to detect differences in cerebral network organizations. Most research has been performed in adults,[22, 23] but also some studies have been performed in children.[24] Although not yet frequently used in the newborn, the first studies have already shown changes in this functional network with increasing maturation.[25-27] It would be of interest to investigate whether this functional network develops differently in infants born preterm in comparison to term born infants and if late-onset sepsis alters the development of the functional network in these infants.

Cerebral imaging offers great opportunities to study the brain in great detail. For example, cerebral tractography might provide indirect information on cerebral white matter tracts. Previous studies demonstrated acute network injury in (term born) infants with neonatal stroke.[28] Also in preterm infants tractography has proven its potential to estimate the integrity of the neonatal brain.[29] The combination of imaging of cerebral tracts on MRI and functional network connectivity examinations by EEG might provide more comprehension about the developing brain.

Final conclusions

In this thesis possible predictors for long term neurodevelopmental outcome were studied. Of course our selection of risk factors is limited and several other factors may play a role in the pathophysiologic processes leading to an adverse outcome in newborn infants, both term and preterm. Further research should elucidate the underlying processes to understand the aberrant development of the neonatal brain, and hopefully provide possibilities to intervene and prevent the detrimental effects of adverse events in the neonatal period.

This can include both pathophysiological pathways in terms of release of inflammatory mediators, as well as vital parameters like cerebral oxygenation measured by NIRS.

Another challenge is a reliable prediction of long term outcome. Many modalities are available already, but new modalities like functional network measurements and tractography may possibly allow us to gain insight in the development and function of the brain. Hopefully with this additional functional information, in the near future we can acquire a more thorough insight in the vulnerable newborn brain.

References

1. Wyatt JS. Mechanisms of brain injury in the newborn. Eye (Lond). 2007;21(10):1261-3.
2. Okereafor A, Allsop J, Counsell SJ, et al. Patterns of brain injury in neonates exposed to perinatal sentinel events. Pediatrics. 2008;121(5):906-14.
3. Jacobs SE, Berg M, Hunt R, et al. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev. 2013(1):CD003311.
4. Debillon T, Bednarek N, Ego A, et al. LyTONEPAL: long term outcome of neonatal hypoxic encephalopathy in the era of neuroprotective treatment with hypothermia: a French population-based cohort. BMC Pediatr. 2018;18(1):255.
5. Tay VY, Bolisetty S, Bajuk B, et al. Admission temperature and hospital outcomes in extremely preterm infants. J Paediatr Child Health. 2018.
6. Alderliesten T, Dix L, Baerts W, et al. Reference values of regional cerebral oxygen saturation during the first 3 days of life in preterm neonates. Pediatr Res. 2016;79(1-1):55-64.
7. Chau V, Brant R, Poskitt KJ, et al. Postnatal infection is associated with widespread abnormalities of brain development in premature newborns. Pediatr Res. 2012;71(3):274-9.
8. Glass HC, Bonifacio SL, Chau V, et al. Recurrent postnatal infections are associated with progressive white matter injury in premature infants. Pediatrics. 2008;122(2):299-305.
9. Perlman J. White matter injury in the preterm infant: an important determination of abnormal neurodevelopmental outcome. Early Human Development. 1998;53(2):99-120.
10. Saliba E, Marret S. Cerebral white matter damage in the preterm infant: pathophysiology and risk factors. Semin Neonatol. 2001;6(2):121-33.
11. Alshaikh B, Yee W, Lodha A, et al. Coagulase-negative staphylococcus sepsis in preterm infants and long-term neurodevelopmental outcome. J Perinatol. 2014;34(2):125-9.
12. Mittendorf R, Covert R, Kohn J, et al. The Association of Coagulase-Negative Staphylococci Isolated from the Chorioamnion at Delivery and Subsequent Development of Cerebral Palsy. J Perinatol. 2001;1:3-8.
13. Zhang Z, Narayan S, Su L, et al. Cerebellar injury and impaired function in a rabbit model of maternal inflammation induced neonatal brain injury. Neurobiol Learn Mem. 2018.
14. Schmidt AF, Kannan PS, Chougnet CA, et al. Intra-amniotic LPS causes acute neuroinflammation in preterm rhesus macaques. J Neuroinflammation. 2016;13(1):238.
15. Patra A, Huang H, Bauer JA, et al. Neurological consequences of systemic inflammation in the premature neonate. Neural Regen Res. 2017;12(6):890-6.
16. Yoon BH, Jun JK, Romero R. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1b, and tumor necrosis factor-a, neonatal brain white matter kesions, and cerebral palsy. Am J Obstet Gynecol. 1997;177:19-26.

17. Kapitanovic Vidak H, Catela Ivkovic T, Vidak Z, et al. COX-1 and COX-2 polymorphisms in susceptibility to cerebral palsy in very preterm infants. Mol Neurobiol. 2017;54(2):930-8.
18. Zarrei M, Fehlings DL, Mawjee K, et al. De novo and rare inherited copy-number variations in the hemiplegic form of cerebral palsy. Genet Med. 2018;20(2):172-80.
19. van Well GTJ, Daalderop LA, Wolfs T, et al. Human perinatal immunity in physiological conditions and during infection. Mol Cell Pediatr. 2017;4(1):4.
20. Lu L, Claud EC. Intrauterine Inflammation, Epigenetics, and Microbiome Influences on Preterm Infant Health. Curr Pathobiol Rep. 2018;6(1):15-21.
21. Cao M, Huang H, Peng Y, et al. Toward Developmental Connectomics of the Human Brain. Front Neuroanat. 2016;10:25.
22. Yu M, Gouw AA, Hillebrand A, et al. Different functional connectivity and network topology in behavioral variant of frontotemporal dementia and Alzheimer's disease: an EEG study. Neurobiol Aging. 2016;42:150-62.
23. Engels MM, Stam CJ, van der Flier WM, et al. Declining functional connectivity and changing hub locations in Alzheimer's disease: an EEG study. BMC Neurol. 2015;15:145.
24. Boersma M, Kemner C, Reus MAd, et al. Disrupted functional brain networks in autistic toddlers. Brain Connectivity. 2013;3:41-9.
25. van de Pol LA, van 't Westende C, Zonnenberg I, et al. Strong Relation Between an EEG Functional Connectivity Measure and Postmenstrual Age: A New Potential Tool for Measuring Neonatal Brain Maturation. Front Hum Neurosci. 2018;12:286.
26. Gonzalez JJ, Manas S, De Vera L, et al. Assessment of electroencephalographic functional connectivity in term and preterm neonates. Clin Neurophysiol. 2011;122(4):696-702.
27. Meijer EJ, Hermans KH, Zwanenburg A, et al. Functional connectivity in preterm infants derived from EEG coherence analysis. Eur J Paediatr Neurol. 2014;18(6):780-9.
28. Dudink J, Counsell SJ, Lequin MH, et al. DTI reveals network injury in perinatal stroke. Arch Dis Child Fetal Neonatal Ed. 2012;97(5):F362-4.
29. Pieterman K, Plaisier A, Govaert P, et al. Data quality in diffusion tensor imaging studies of the preterm brain: a systematic review. Pediatr Radiol. 2015;45:1372-81.

Summary

Summary

In this thesis several predictors for neurodevelopmental outcome in term and preterm infants were studied. These predictors may occur during birth, but also in the postnatal period.

Chapter 1 provides information about the neuro-ontogenesis of the human brain. Throughout gestation the brain develops rapidly, thereby opposing a risk of aberrant development when unexpected events occur. Several modalities to gain insight in adverse effects of these events and implications on long term outcome have been described.

The aim and outline of this thesis are discussed.

Part 1: Perinatal predictors

Chapter 2 describes the effect of acute neonatal anaemia on MRI findings in the neonatal period and the neurodevelopmental outcome. A distinction has been made between newborns who encountered mainly acute anaemia versus newborns experiencing perinatal asphyxia as well. This study shows that severe neonatal anaemia is associated with high neonatal morbidity and mortality. White matter damage was the main pattern of brain injury found on cerebral MRI imaging. However, it is difficult to distinguish between the effects of neonatal anaemia only and of perinatal asphyxia, which was present in this cohort as well.

Chapter 3 addresses the differences in long term neurodevelopmental outcome at four years of age in children who suffered from perinatal asphyxia using the Ages and Stages Questionnaire. Children treated with therapeutic hypothermia were compared to children who did not receive therapeutic hypothermia. The overall mean scores did not differ significantly between the groups. However, the normothermic group scores were more frequently below -2 SD, compared to the hypothermic group.

Part 2: Postnatal predictors

In this part several modalities to predict outcome are described. This part covers a cohort of preterm infants with a gestational age <32 weeks and/or <1500 grams suspected of late-onset sepsis.

In *chapter 4* a pilot study has been performed to investigate the effects of late-onset sepsis during the first 72 hours after onset, on cerebral oxygenation using Near InfraRed

Spectroscopy (NIRS). In this cohort, late-onset sepsis was mainly caused by coagulase negative staphylococci (CoNS). No association was found between the occurrence of late-onset sepsis and aberrant NIRS values. Moreover, those with a late-onset sepsis showed no impaired neurodevelopmental outcome at the corrected age of two years.

In *chapter 5* the findings on subsequent cerebral ultrasound imaging during late-onset sepsis are reported. In this cohort no differences in major brain abnormalities were found. Moreover, two third of the proven infections were caused by CoNS. Only three out of 66 infants showed major abnormalities on cerebral ultrasound, which might suggest a minor role on at least the short term effects of late-onset sepsis caused by CoNS. In two of the twelve patients infected with *Staphylococcus Aureus*, cerebral ultrasound showed abnormalities in the thalamus.

Chapter 6 addresses early motor outcome of this cohort using Alberta Infant Motor Scale (AIMS). A significant difference was found between the groups with and without late-onset sepsis in favor of the sepsis group. Deterioration in the AIMS score at twelve months corrected age compared to the scores at six months corrected age were found in the late-onset sepsis group. This finding might indicate that late-onset sepsis might be a factor in development of gross motor developmental delay. In subgroup analyses at six months corrected age a trend to lower AIMS was found in patients with necrotizing enterocolitis compared to those without necrotizing enterocolitis.

Chapter 7 describes the neurodevelopmental outcome of this cohort at two years corrected age using the Bayley's Scales of Infant Development (BSID), version II. At two years corrected age no differences in neurodevelopmental outcome were observed between the proven late-onset sepsis group versus no proven sepsis group. Preterm infants encountering late-onset sepsis by CoNS in comparison to other causal infectious agents show a better cognitive outcome as tested by BSID version II.

Chapter 8 summarizes the findings of this thesis and discusses future perspectives to gain more insight in the pathophysiology of adverse events during birth and in the postnatal period. Also new modalities are discussed, which might provide information of expected neurodevelopmental outcome and thereby could be helpful to predict long term neurological outcome in the vulnerable newborn admitted to the Neonatal Intensive Care Unit.

Nederlandse samenvatting

Nederlandse samenvatting

In dit proefschrift worden gebeurtenissen bestudeerd die van invloed zouden kunnen zijn op de neurologische ontwikkeling in prematuur en à terme geboren kinderen. Deze gebeurtenissen kunnen voorspellers zijn en kunnen zich voordoen rondom de geboorte, maar ook later in de postnatale periode.

Hoofdstuk 1 beschrijft de neuro-ontogenese van de neonatale hersenen. Gedurende de zwangerschap ontwikkelen de hersenen zich snel. Zodoende ontstaat het risico op een afwijkende ontwikkeling wanneer onverwachte gebeurtenissen zich voordoen. Verschillende modaliteiten om inzicht te verwerven in de negatieve effecten van gebeurtenissen en de implicaties op de lange termijn psychomotore ontwikkeling worden beschreven.

Het doel en de inhoud van dit proefschrift wordt in dit hoofdstuk besproken.

Deel 1: Perinatale voorspellers

Hoofdstuk 2 beschrijft de effecten van acute neonatale anemie op het patroon van neonatale hersenschade, aan de hand van MRI beelden van de hersenen in relatie tot de psychomotore ontwikkeling. We hebben een onderscheid gemaakt tussen pasgeborenen die voornamelijk de gevolgen van acute anemie hadden doorgemaakt en pasgeborenen die tevens een perinatale asfyxie hadden doorgemaakt. Deze studie laat zien dat ernstige neonatale anemie is geassocieerd met hoge neonatale morbiditeit en mortaliteit. Het meest voorkomende patroon van hersenschade gevonden op cerebrale MRI was witte stof schade. Echter, het is niet goed mogelijk te differentiëren tussen het effect van neonatale anemie alleen en bijkomende effecten van perinatale asfyxie, die bij deze kinderen ook zeker een rol speelde.

Hoofdstuk 3 adresseert de verschillen tussen kinderen die behandeld waren met therapeutische hypothermie versus kinderen zonder therapeutische hypothermie. De lange termijn psychomotore uitkomst werd op de leeftijd van vier jaar onderzocht gebruikmakend van de Ages and Stages Questionnaire. De totale gemiddelde score van de groep kinderen met en zonder therapeutische hypothermie waren niet verschillend. Echter, in de normothermie groep waren de scores vaker onder de -2 standaard deviatie in vergelijking middels de met hypothermie behandelde groep. Met andere woorden de kinderen die niet gekoeld waren hadden vaker een slechte psychomotore ontwikkeling dan de kinderen die wel gekoeld waren.

Deel 2: Postnatale voorspellers

In dit deel van het proefschrift worden verschillende modaliteiten beschreven om de (lange termijn) uitkomst te voorspellen. Dit deel beschrijft een cohort van prematuur geboren kinderen met een zwangerschapsduur van minder dan 32 weken en/of een geboortegewicht lager dan 1500 gram die verdacht worden van sepsis meer dan 72 uur na geboorte (late-onset sepsis).

In *hoofdstuk 4* werd een pilotstudie verricht waarin gedurende de eerste 72 uur na het stellen van de diagnose late-onset sepsis de effecten van deze sepsis op de cerebrale oxygenatie middels Near InfraRed Spectroscopy (NIRS) werden bestudeerd. In dit cohort werd de late-onset sepsis voornamelijk veroorzaakt door coagulase negatieve staphylococcen (CNS). Er werd geen associatie gevonden tussen het doormaken van een sepsis en afwijkende NIRS waarden. Bovendien werd bij hen geen afwijkende neurologische ontwikkeling aangetoond op de gecorrigeerde leeftijd van twee jaar.

In *hoofdstuk 5* worden de bevindingen van herhaaldelijk echografisch onderzoek van de hersenen ten tijde van late-onset sepsis beschreven. In dit cohort werden geen verschillen in grote hersenafwijkingen gevonden. In twee derde van de bewezen infecties werd de sepsis veroorzaakt door CNS. Slechts drie van de 66 kinderen met een CNS sepsis toonden grote afwijkingen bij echografisch onderzoek van de hersenen, hetgeen zou kunnen suggereren dat er slechts een geringe rol is weggelegd voor tenminste de korte termijn effecten van een CNS sepsis. Bij twee van de twaalf kinderen met een Staphylococcus Aureus sepsis werden echografische afwijkingen in de thalamus gevonden.

Hoofdstuk 6 beschrijft de vroeg motorische uitkomst van dit cohort, onderzocht met de Alberta Infant Motor Scale (AIMS). Op de leeftijd van zes maanden werd er een verschil aangetoond tussen de groepen met en zonder late-onset sepsis in het voordeel van de kinderen die een sepsis hadden doorgemaakt. Echter op de leeftijd van twaalf maanden werd er in de sepsis groep een verslechtering van de AIMS-scores gezien. Mogelijk geeft deze bevinding aan dat late-onset sepsis een rol speelt in de latere grof-motorische ontwikkeling. In een subgroep analyse van kinderen met necrotiserende enterocolitis werd er op de gecorrigeerde leeftijd van zes maanden een trend in lagere AIMS scores aangetoond ten opzichte van kinderen die geen necrotiserende enterocolitis ontwikkelden.

Hoofdstuk 7 beschrijft de psychomotore ontwikkeling van dit cohort op de gecorrigeerde leeftijd van twee jaar, waarbij de ontwikkeling werd bestudeerd middels de Bayley Scales of Infant Development (BSID), versie II. Op de gecorrigeerde leeftijd van twee jaar werden geen verschillen aangetoond in de psychomotore ontwikkeling tussen de groep met een bewezen late-onset sepsis en de groep zonder een bewezen infectie. Prematuur geboren kinderen die een late-onset sepsis doormaakten veroorzaakt door CNS lieten met de BSID versie II test een betere cognitieve ontwikkeling zien in vergelijking met kinderen die een sepsis met een andere verwekker doormaakten.

Hoofdstuk 8 vat de bevindingen van dit proefschrift samen en bespreekt mogelijkheden om in de toekomst meer inzicht te krijgen in de pathofysiologie van gebeurtenissen rondom de geboorte en in de postnatale periode. Ook worden nieuwe modaliteiten besproken, die informatie zouden kunnen geven over de te verwachten psychomotore ontwikkeling. Dit zou behulpzaam kunnen zijn in het voorspellen van lange termijn neurologische uitkomsten van de kwetsbare pasgeborene die eerder was opgenomen op de Neonatale Intensive Care.

Abbreviations

ADC	Apparent diffusion coefficient
AED	Anti-epileptic drugs
aEEG	Amplitude integrated electroencephalography
AIMS	Alberta Infant Motor Scale
AS	Apgar score
ASQ	Ages and Stages Questionnaire
BGT	Basal ganglia and thalami
BPD	Bronchopulmonary dysplasia
BS	Burst suppression
BSID	Bayley Scales of Infant and toddler Development
BSID-II	Bayley Scales of Infant and toddler Development, second version
BSID-III	Bayley Scales of Infant and toddler Development, third version
BSID-II-NL	Bayley Scales of Infant and toddler Development, second version, Dutch edition
BW	Birth weight
CA	Cortex abnormalities
CBCL	Child Behavior Checklist
cFTOE	Cerebral fractional tissue oxygen extraction
CNV	Continuous normal voltage
Com	Communication score
CoNS	Coagulase negative staphylococcus
COX	Cyclooxygenase
CP	Cerebral palsy
CRIB	Clinical risk index for babies
CRP	C-reactive protein
DNV	Discontinuous normal voltage
DWI	Diffusion weighted images
EA	Epileptic activity
EEG	Electroencephalography
FMT	Foetomaternal transfusion
F	Female
FM	Fine motor skills
FT	Flat trace
GA	Gestational age
GBS	Group B streptococcus
GM	Gross motor skills
GMDS	Griffiths Scale of Mental Development

Hb	Hemoglobin
HELLP	Hemolysis elevated liver enzymes and low platelets
HS-PDA	Hemodynamic significant ductus arteriosus
HT	Hypothermia
IC	Internal capsule
IL	Interleukin
IVH	Intraventricular hemorrhage
LDQ	Locomotor developmental index
LOS	Late-onset sepsis
M	Male
MDI	Mental developmental index
MRI	Magnetic resonance imaging
NA	No abnormalities
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
NIRS	Near infrared spectroscopy
NP	Not performed
PCR	Polymerase chain reaction
PHVD	Post hemorrhagic ventricular dilatation
PDA	Patent ductus arteriosus
PDI	Psychomotor developmental index
PDQ	Performance developmental index
PE	Psycho-emotional
PS	Problem solving
Pt	Patient
PVL	Periventricular leukomalacia
PWML	Punctate white matter lesions
RDS	Respiratory distress syndrome
$rScO_2$	Regional cerebral oxygen saturation
S	Stroke
SaO_2	Arterial oxygen saturation
SD	Standard deviation
TLR	Toll like receptor
TNF	Tumor necrosis factor
UC pH	Umbilical cord pH
WM	White matter
WMI	White matter injury

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Publications

International

Zonnenberg IA, van den Dungen FAM, Vermeulen RJ, Schaaf VAM, van Weissenbruch MM. Motor outcome in preterm infants with and without nosocomial infection during NICU admittance at 6 and 12 months corrected age. *Submitted*.

Favié LMA, Groenendaal F, van den Broek MPH, Rademaker CMA, de Haan TR, van Straaten HLM, Dijk PH, van Heijst A, Simons SHP, Dijkman KP, Rijken M, **Zonnenberg IA**, Cools F, Zecic A, van der Lee JH, Nuytemans DHGM, van Bel F, Egberts TCG, Huitema ADR, on behalf of the PharmaCool study group. Phenobarbital, midazolam pharmacokinetics, effectiveness and drug-drug interaction in asphyxiated neonates undergoing therapeutic hypothermia. Neonatology. *Accepted for publication*.

Zonnenberg IA, van Dijk J, van den Dungen FAM, Vermeulen RJ, van Weissenbruch MM. The prognostic value of NIRS in preterm infants with (suspected) late-onset sepsis: a pilot study. PLoS One. 2019 Jul 24;14(7):e0220044.

Zonnenberg IA, van Dijk-Lokkart E, van den Dungen FAM, Vermeulen RJ, van Weissenbruch MM. Neurodevelopmental outcome at two years of age in preterm infants with late-onset sepsis. European Journal of Pediatrics. Eur J Pediatr. 2019;178(5):673-80.

Favié LMA, Groenendaal F, van den Broek MPH, Rademaker CMA, de Haan TR, van Straaten HLM, Dijk PH, van Heijst A, Dudink J, Dijkman KP, Rijken M, **Zonnenberg IA**, Cools F, Zecic A, van der Lee JH, Nuytemans DHGM, van Bel F, Egberts TCG, Huitema ADR. Pharmacokinetics of morphine in encephalopathic neonates treated with therapeutic hypothermia. PLoS One. 2019;14(2):e0211910.

Onland W, Cools F, Kroon A, Rademaker K, Merkus M, Dijk P, van Straaten H, te Pas A, Mohns T, Theyskens C, van Heijst A, Kramer B, Debeer A, **Zonnenberg I**, Marechal Y, Blom H, Plaskie K, Offringa M, van Kaam A, for the STOP-BPD study group. Effect of hydrocortisone therapy initiated 7-14 days after birth on mortality or bronchopulmonary dysplasia among very preterm infants receiving mechanical ventilation. A randomized clinical trial. JAMA. 2019;321(4):354-63.

Hakobyan M, Dijkman KP, Laroche S, Naulaers G, Rijken M, Steiner K, van Straaten HLM, Swarte RMC, ter Horst HJ, Zecic A, **Zonnenberg IA**, Groenendaal F. Outcome of infants with therapeutic hypothermia after perinatal asphyxia and early onset sepsis. Neonatology. 2018;115(2):127-33.

Pouwels PJW, van de Lagemaat M, van de Pol LA, Witjes BCM, **Zonnenberg IA**. Spectroscopic detection of brain propylene glycol in neonates: effects of different pharmaceutical formulations of phenobarbital. J Magn Reson Imaging. 2019;49(4):1062-8.

van der Knoop BJ, van der Voorn JP, Nikkels PGJ, **Zonnenberg IA**, van Weissenbruch MM, Vermeulen RJ, de Vries JIP. Placental histology after minor trauma in pregnancy, a pilot study. Pediatr Dev Pathol. 2019;22(3):221-8.

Van de Pol LA, van 't Westende C, **Zonnenberg IA**, Koedam ELGE, van Rossum IA, de Haan W, Steenweg ME, van Straaten ECW, Stam CJ. Strong relation between an EEG functional connectivity measure and postmenstrual age: A new potential tool for measuring neonatal brain maturation. Front Hum Neurosci. 2018;12:286.

Van der Knoop BJ, Oostrom K, **Zonnenberg IA**, van Weissenbruch MM, Vermeulen RJ, de Vries JIP. Neurobehavioural outcome in 6-18 year old children after trauma in pregnancy: Case-control study. Eur J Paediatr Neurol. 2018;22(5):845-53.

Bijleveld YA, de Haan TR, van der Lee JH, Groenendaal F, Dijk PH, van Heijst A, de Jonge RCJ, Dijkman KP, van Straaten HLM, Rijken M, **Zonnenberg IA**, Cools F, Zecic A, Nuytemans DHGM, van Kaam AH, Mathôt RAA; PharmaCool Study Group. Evaluation of a system-specific function to describe the pharmacokinetics of benzylpenicillin in term neonates undergoing moderate hypothermia. Antimicrob Agents Chemother. 2018;62(4).

Bijleveld YA, Mathôt R, van der Lee JH, Groenendaal F, Dijk PH, van Heijst A, Simons S, Dijkman KP, van Straaten H, Rijken M, **Zonnenberg IA**, Cools F, Zecic A, Nuytemans D, van Kaam AH, de Haan TR. Population pharmacokinetics of amoxicillin in term neonates undergoing moderate hypothermia. Clin Pharmacol Ther. 2018;103(3):458-67.

Van der Knoop BJ, **Zonnenberg IA**, Otten VM, van Weissenbruch MM, de Vries JIP. Trauma in pregnancy, obstetrical outcome in a tertiary centre in the Netherlands. J Matern Fetal Neonatal Med. 2018;31(3):339-46.

Claessens LC*, **Zonnenberg IA***, van den Dungen FAM, Vermeulen RJ, van Weissenbruch MM. *contributed equally. Cerebral ultrasound abnormalities in preterm infants caused by late-onset sepsis. PLoS One. 2017;12(3):e0173227.

Zonnenberg IA, Koopman C, van Schie PE, Vermeulen RJ, Groenendaal F, van Weissenbruch MM. Comparison of psychomotor outcome in patients with perinatal asphyxia with versus without therapeutic hypothermia at 4 years using the Ages and Stages Questionnaire screening tool. Eur J Paediatr Neurol. 2016;20(4):545-8.

Thorsen P, Jansen-van der Weide MC, Groenendaal F, Onland W, van Straaten HL, **Zonnenberg I**, Vermeulen JR, Dijk PH, Dudink J, Rijken M, van Heijst A, Dijkman KP, Cools F, Zecic A, van Kaam AH, de Haan TR. The Thompson Encephalopathy Score and Short-Term Outcomes in Asphyxiated Newborns Treated With Therapeutic Hypothermia. Pediatr Neurol. 2016;60:49-53.

Zonnenberg IA, Vermeulen RJ, Rohaan MW, van Weissenbruch MM, Groenendaal F, de Vries LS. Severe neonatal anaemia, MRI findings and neurodevelopmental outcome. Neonatology. 2016;109(4):282-8.

Bijleveld YA, de Haan TR, van der Lee JH, Groenendaal F, Dijk PH, van Heijst A, de Jonge RC, Dijkman KP, van Straaten HL, Rijken M, **Zonnenberg IA**, Cools F, Zecic A, Nuytemans D, van Kaam AH, Mathot RA; PharmaCool study group. Altered gentamicin pharmacokinetics in term neonates undergoing controlled hypothermia. Br J Clin Pharmacol. 2016;81(6):1067-77.

Bijleveld Y, de Haan T, Toersche J, Jorjani S, van der Lee J, Groenendaal F, Dijk P, van Heijst A, Gavilanes AW, de Jonge R, Dijkman KP, van Straaten H, Rijken M, **Zonnenberg I**, Cools F, Nuytemans D, Mathôt R. A simple quantitative method analysing amikacin, gentamycin, and vancomycin levels in human newborn plasma using ion-pair liquid chromatography/tandem mass spectrometry and its applicability to a clinical study. J Chromatogr B Analyt Technol Biomed Life Sci. 2014;951-952:110-8.

Groenendaal F, Casaer A, Dijkman KP, Gavilanes AWD, de Haan TR, ter Horst HJ, Laroche S, Naulaers G, Rijken M, van Straaten HLM, Steiner K, Swarte RMC, Zecic A, **Zonnenberg IA**, and the Dutch-Flemish Working Group on Neonatal Neurology. Introduction of Hypothermia for Neonates with Perinatal Asphyxia in the Netherlands and Flanders. Neonatology. 2013;104:15-21.

van der Knoop BJ, de Vries JIP, **Zonnenberg IA**, Verbeke JIML, Vermeulen RJ. Tutorial on neonatal cranial ultrasound. Online publication European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) June 5, 2013. https://issuu.com/efsumb/docs/neonatal__tutorial_final

Zonnenberg I, De Waal KA. The definition of a haemodynamic significant duct in randomized controlled trials: a systematic literature review. Acta Paediatr. 2012;101(3):247-251.

Lakkundi A, de Waal K, **Zonnenberg I**. 636 Quantified Evaluation of the Ductus Arteriosus. Pediatr Res. 2010;68:325.

Vanhaesebrouck S, **Zonnenberg I**, Vandervoort P, Bruneel E, Van Hoestenberghe MR, Theyskens C. Conservative treatment for patent ductus arteriosus in the preterm. Arch Dis Child Fetal Neonatal Ed. 2007;92(4):F244-7.

National

Ten Hove CH, Vermeulen RJ, **Zonnenberg IA**. Tweedegeneratie-anti-epileptica voor behandeling van neonatale epileptische aanvallen. Praktische Pediatrie. September 2015.

Zonnenberg IA, van der Lee JH, Kok JH, Sauerwein HP, van Kempen AAMW. Lage insulinewaarden bij pasgeborenen rond de à terme datum. Tijdschr Kindergeneeskd. 2014;82(3):112-6.

Groenendaal F, Casaer A, Dijkman KP, Gavilanes AWD, de Haan TR, ter Horst HJ, Laroche S, Naulaers G, Rijken M, van Straaten HLM, Steiner K, Swarte RMC, Zecic A, **Zonnenberg IA**. Therapeutische hypothermie bij perinatale asfyxie. Kinderarts en Wetenschap. Maart 2012.

Zonnenberg IA, Semmekrot BA. Neonatale polycythemie: voorbeelden uit de praktijk en overzicht van de literatuur. Tijdschr Kindergeneeskd. 2005;73(2):74-7.

Zonnenberg IA, van der Staak FH, Cuppen MP, Zwart A. Dumpingsyndroom bij een kind met een verkeerd gelegen gastrostomiekatheter. Ned Tijdschr Geneeskd. 2003;147(8):350-3.

Curriculum Vitae

Inge Zonnenberg was born on September 14, 1975 in Oss. In 1993 she finished secondary school at the Maasland College in Oss. She graduated medical school at Radboud University, Nijmegen in 1999. Following graduation she first worked as resident at Slingeland Ziekenhuis, Doetinchem and later at Canisius Wilhelmina Ziekenhuis, Nijmegen. Thereafter she started her residency in Pediatrics in Belgium at the Vrije Universiteit Brussel, continued at Queen Paola's Children's Hospital in Antwerp, and Ziekenhuis Oost Limburg in Genk. The last two years of her training was at Wilhelmina's Children's Hospital in Utrecht which she finished 2006. Subsequently she started her fellowship in neonatology at the Academic Medical Center in Amsterdam which she completed in 2009. She continued working as neonatologist and transferred to the VU Medical center in February 2010. She has been working there since and started with the research which resulted in this thesis. In 2018 she graduated her master degree in Clinical Epidemiology.

She is married to Remco van Rees. They have two sons, Thijs and Jasper, and live in Amersfoort.

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Kijkend op de voorbije jaren, is het niet altijd gemakkelijk geweest, maar dankzij jullie allemaal heb ik dit proefschrift tot een goed einde gebracht! Dank hiervoor!